Artificial hybrids in *Cryptocoryne* (Araceae). 1. Hybridization between Sri Lankan species within the 2*n* = 28 chromosome group.

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ABSTRACT

The Sri Lankan *Cryptocoryne* of the 2*n* = 28 chromosome group are among the most often-cultivated species of aquarium plants but despite this there have been many problems in identifying and delimiting these species. Here we show that hybridization and hybrids are widespread within the group, probably one of the reasons for causing identification problems of the cultivated plants. Artificial hybridization experiments show that the only major hybridization barrier is between *C. nevillii* and *C. parva*, and that the *C. parva* hybrids have a low pollen fertility, while the other Sri Lankan hybrids are fertile.

KEY WORDS

Araceae, chromosome numbers, *Cryptocoryne*, hybrids, natural and artificial hybridization, Sri Lanka

INTRODUCTION

Species of *Cryptocoryne* (Araceae) from Sri Lanka have over the years been treated in various works since the description of the first two species (Schott 1857). An overview of the history, taxonomy and cytology of the Sri Lankan 2*n* = 28 chromosome group may be found in Jacobsen (1976). Further information may be found in Wit (1990) and Bastmeijer (2019).

Table 1. Sri Lankan *Cryptocoryne* species of the $2n = 28$ chromosome group and their general distribution within the island.

C. beckettii Trim.	$2n = 28, 42$	Central
C. undulata Wendt	$2n = 28, 42$	Central
C. walkeri Schott	$2n = 28, 42$	Central
C. wendtii De Wit	$2n = 28, 42$	North (-western)
C. parva De Wit	$2n = 28$	Central
C. nevillii Hook.f.	$2n = 28$	Eastern
C. ×*willisii* Reitz	$2n = 28$	Central

The present study is based on a field trip to Sri Lanka in March 1975, and a subsequent study of the Sri Lankan species of *Cryptocoryne* (Jacobsen 1976), initiating the contribution to the Flora of Ceylon (Jacobsen 1987).

The study of chromosome numbers in *Cryptocoryne* was introduced by Marchant (1972, 1973), Legro (1967, 1971) and continued by Jacobsen (1976, 1977a & b), Arends et al. (1982) and Graaf & Arends (1986). It became clear that chromosome numbers in *Cryptocoryne* were very useful characters. Within Sri Lanka two chromosome groups were found: $2n = 2x = 28$ (and $2n = 3x = 42$) in species related to *C. walkeri* Schott and $2n = 36$ in species related to *C. thwaitesii* Schott.

In the study of chromosome numbers and taxonomy in *Cryptocoryne*, the question of the occurrence of hybrids was put forward and further investigated and supported by artificial hybridization experiments (Jacobsen 1977a & b; 1981a & b; Arends et al. 1982). An overview of the present knowledge of naturally occurring hybrids in the genus *Cryptocoryne* was presented by Jacobsen et al. (2016) and Jacobsen & Ørgaard (2019), providing data on the known naturally occurring hybrids, as well as comments on some of the artificially produced hybrids.

Artificial hybridization

A program of interspecific hybridization in *Cryptocoryne* has taken place over the years at the University of Copenhagen, and Sri Lankan species of the $2n = 28$ group were among the first to be investigated (Jacobsen 1981a & b; 1987; Jacobsen et al. 2016). This was primarily due to the abundance of material available for experiments at that time, in large part due to material commercially exported from Sri Lanka as aquarium plants but also samples obtained from a field trip in 1975 (Jacobsen 1976).

The Sri Lankan $2n = 28$ group consists of six presently-recognized species (Table 1),

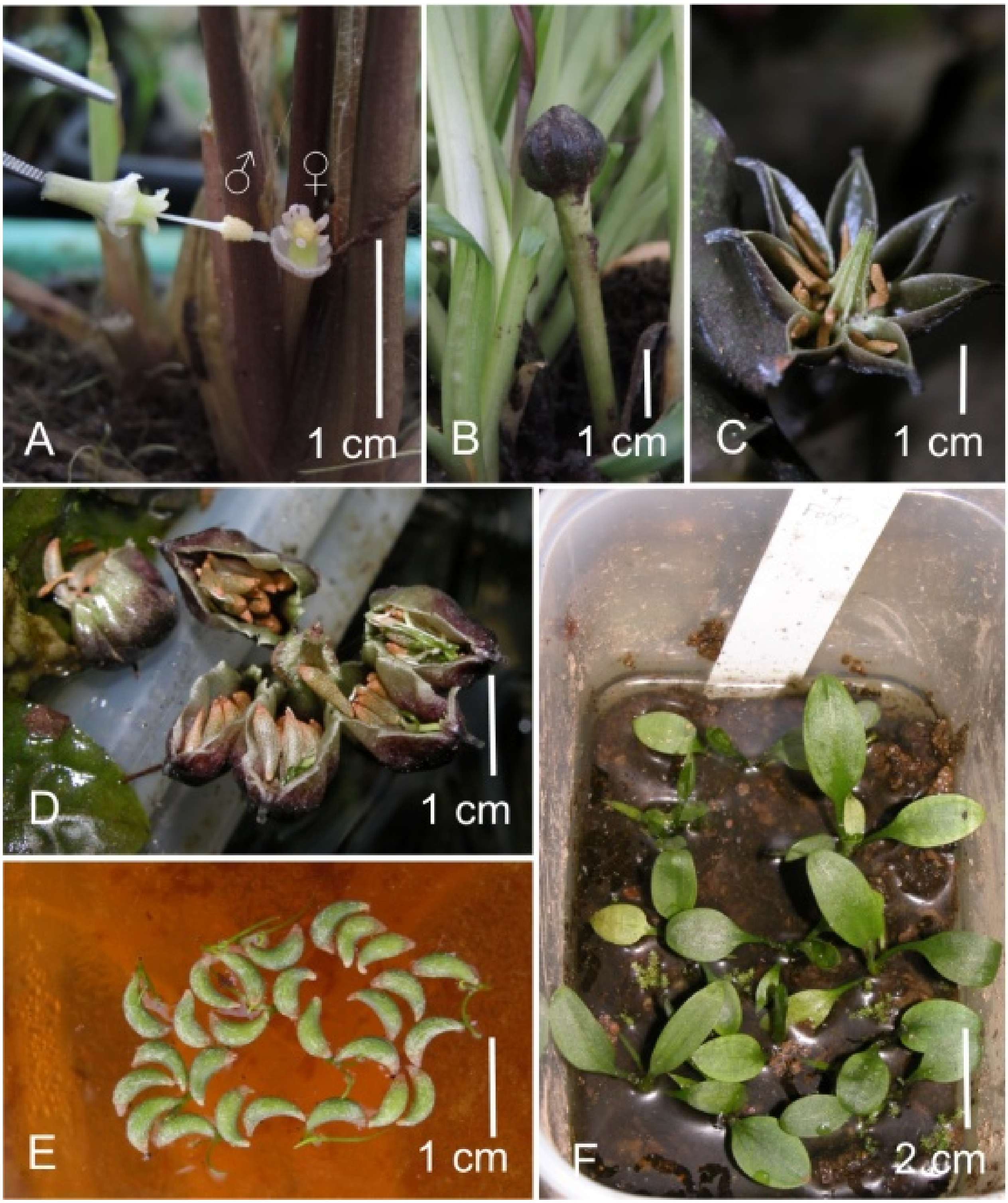

Figure 1. A. Spadix of *Cryptocoryne* at the female stage (right) with the spathe and male flowers cut off (day one) being pollinated by a cut-off spadix with male flowers (day 2–3); **B.** at maturity the infructescence is raised above soil surface by elongation of the peduncle; **C.** Mature, open infructescence exposing the seeds; **D.** fully mature infructescence splitting each fruit from the central peduncle axis; **E.** floating germinating seeds; **F.** seedlings with the first developing leaves.

Table 2. Data on the accessions used in the crossing program.

“*C. beckettii* × *C. walkeri*”, NJ 23-7, Halloluwa, Kandy, 23 March 1975
“*C. beckettii* × *C. walkeri*”, NJ 23-6, Halloluwa, Kandy, 23 March 1975
“*C. beckettii* × *C. walkeri*” / “*walkeri*”, AdG 288, Halloluwa, Kandy, April 1981
C. beckettii NJ 23-9, Halloluwa, Kandy, 23 March 1975
C. beckettii, Kew 334-70-03263, cultivated, 1975
C. beckettii, Jayasuriya 2246, NJ 2942, Rhuna N.P., 16 July 1975
C. nevillii, NJ 3093, Nicholson 4268, 25 km stone, Baticaloa, 15 February 1979
C. nevillii, NJ 3234, Manresa Mission, ‘Tropica’ cultivated, 25 August 1981
C. parva, 1671/16, Windeløv s.n., Halloluwa, Kandy, 20 February 1973
C. parva, NJ 3094, ‘Tropica’ cultivated, 1979
C. undulata, HBH P1961/542, cultivated, 1961
C. undulata, NJ 23-2, Halloluwa, Kandy, 23 March 1975
C. walkeri, NJ 2836, cultivated, c. 1970
C. walkeri, NJ 3182, Windeløv s.n., ‘Tropica’ cultivated, 1982
C. wendtii, Kew 334.70-03260, cultivated, 1975
C. wendtii, NJ 2781, ‘Akvarium København’, cultivated, 1971
C. wendtii NJ 2793, ‘Planteimporten’, cultivated, 1972
C. wendtii, NJ 2824, ’Akvarium København’, cultivated, 1972
C. wendtii, NJ 3216, Windeløv s.n., Malvatu Oya, 1980
C. wendtii, NJ 3237,’Tropica’ cultivated, 1982

of which some also are represented by triploid types ($2n = 3x = 42$), and two naturally occurring diploid hybrids, *C.* ×*willisii* and *C. beckettii* × *C. walkeri*.

A main point of interest with artificial crossing was to see 1) whether it was possible to resynthesize the naturally-occurring hybrid combinations, 2) to see how many of the potential hybrid combinations it was possible to obtain, 3) and observe the result of successful combinations.

Throughout this paper the female parent in artificial crosses is placed first; when the paternity is unknown the parents are listed in alphabetical order.

Table 3. Crossing attempts with the Sri Lankan *Cryptocoryne* of the $2n = 28$ group with number of crossing attempts.

Total number of attempts:	264
$2n = 28 \times 2n = 28$:	130
$2n = 28 \times 2n = 3x = 42$:	10
C. ×*willisii* × $2n = 28$:	8
C. ×*willisii* × *C.* ×*willisii*:	5
$2n = 28$ × out of group:	28
NJ 23-6, -7 and Similar F_1	23
Complex hybrids (3 or more):	60

Four groups of triploids have been referred to the morphologically most similar diploid species, but only morphological characters have been used to support such a grouping. The possibility of the triploids hitherto referred to *C. beckettii*, *C. undulata*, and *C. walkeri* actually being interspecific hybrids could be very high, while the triploids referred to *C. wendtii* might be of intraspecific origin, as these are geographically separated from the other species. A molecular study of these complexes might elucidate these questions.

MATERIALS AND METHODS

Parental material

Data on the origin of parents of the hybrids is provided in **Table 2**. A few accessions included provided some identification and taxonomic problems which will be treated in relation to the relevant crossings.

Crossing methods

For the crossings, spathes were used as females at the first day of opening. A small horizontal incision was made at the base of the kettle. The first day female spathes have a distinct crispness, so the whole spathe can be snapped off at the incision. The spathe comes off together with the male portion of the spadix which then is firmly attached to the spathe by the spadix appendix: the spadix breaks at the basis of the thin axis thereby exposing the female flowers.

Two to three-day old spathes, in which the male flowers had matured, were cut off and used as males. As with the spathes to be used as females, a small incision was made at the base of the kettle and the upper part pulled off. At this time the male flowers and

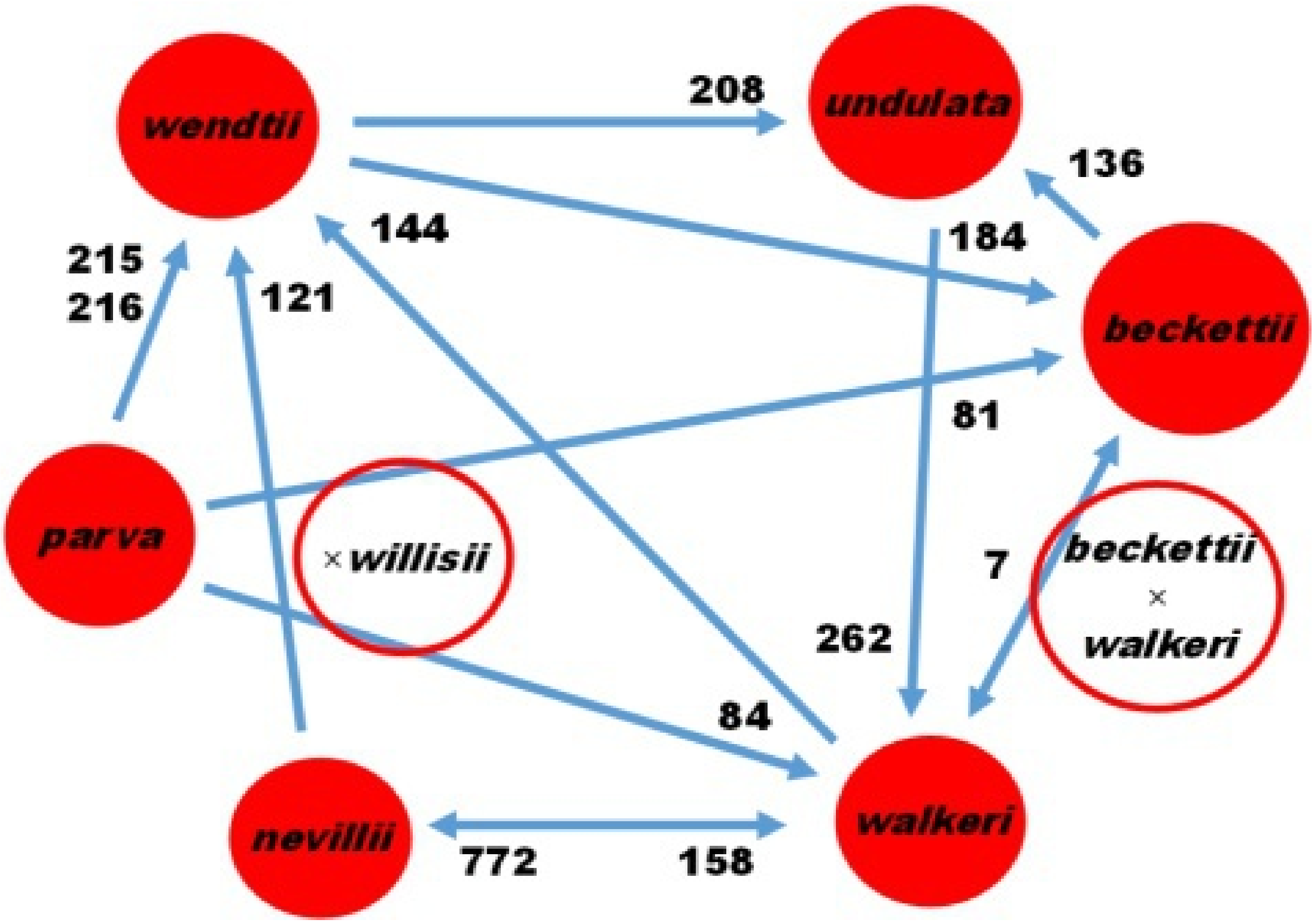

Figure 2. A crossing polygon showing the six Sri Lankan 2n = 28 chromosome *Cryptocoryne* species (solid circles) and the obtained combinations. The natural hybrids are marked by a red, open circle. Of the 15 possible combinations it has been possible to obtain 12 combinations. The numbers in the figure refers to the obtained hybrid CyCy combinations as referred to in the text and the figures. The arrows point towards the female parent.

the sterile spadix appendix are no longer attached to the spathe but by the thin sterile part rising from the center of the female flower zone. By holding the exposed spadix by the female part, the whole spadix can be maneuvered so the male part functions as a “brush” by which one can apply the pollen droplets to the receptive stigmas (**Figure 1**).

The pollinated flowers/plants were returned into their original high humidity growing conditions. If pollination was successful, the female infructescence began to grow in size after a few weeks. The infructescence matured within 4–8 months after which period the peduncle elongated by several cm; the fruit opened in a star-like manner, exposing the seeds. The seeds were either floated on water until germination

Figure 3. CyCy 7 cross. **A ♀.** NJ 23–7, Halloluwa; **B ♂.** NJ 23–7, Halloluwa; **C – J.** Hybrid siblings representing CyCy 7: ["*C. beckettii* × *C. walkeri*"] × ["*C. beckettii* × *C. walkeri*"]. NJ 23–7 is a naturally-occurring fertile hybrid between *C. beckettii* and *C. walkeri*.

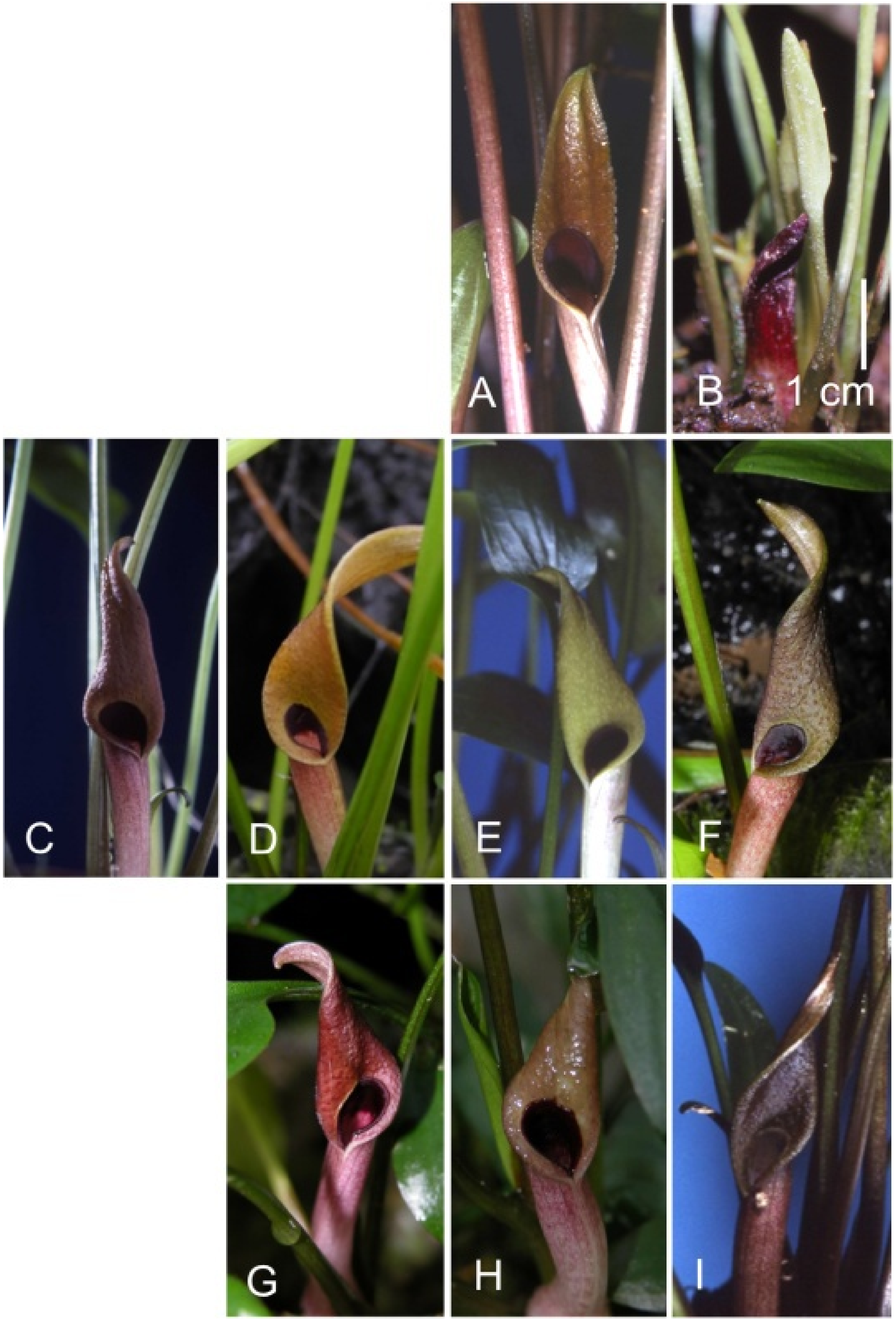

Figure 4. CyCy 81 cross. **A** ♀. NJ 23–9, Halloluwa; **B** ♂. NJ 3094, cult.; **C – I.** Hybrid siblings representing CyCy 81: *C. beckettii* × *C. parva.*

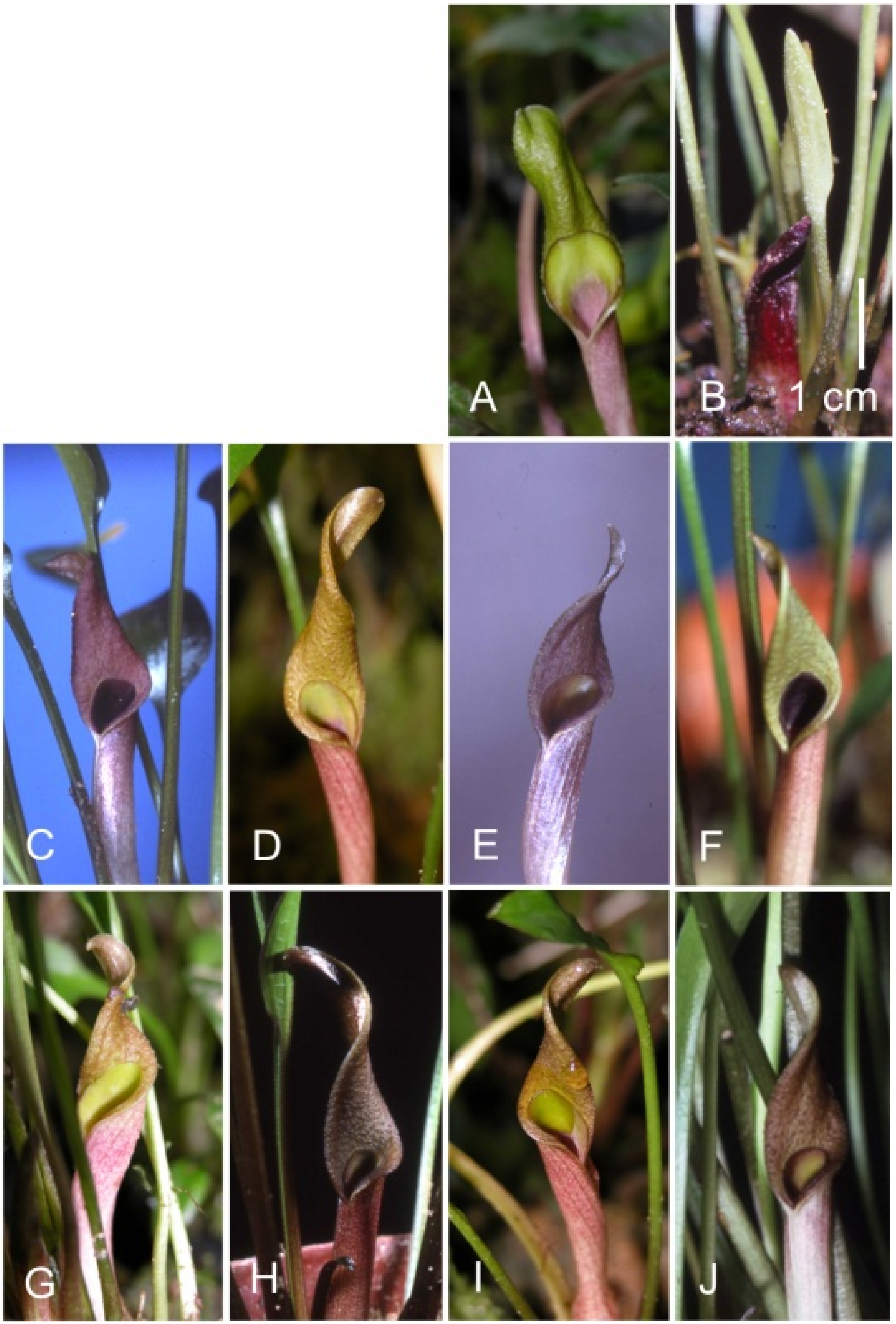

Figure 5. CyCy 84 cross. **A** ♀. NJ 23–7, Halloluwa; **B** ♂. NJ 3094, cult.; **C – J**. Hybrid siblings representing CyCy 84: [*C. beckettii* × *C. walkeri*] × *C. parva*.

within a few days or placed directly on wet soil. After attaining a reasonable size, the seedlings were transferred to pots where they remained until flowering.

When the infructescence was left on the plant to mature fully, the seeds of interspecific crosses sometimes aborted at a later stage, and it was our experience that it could be advantageous to extract the seeds before they were fully mature, as this seems to maximize the probability of germination.

Flowering of the hybrids took place the year after germination or later, depending on the growing conditions.

General aspects of hybridization within *Cryptocoryne*

In interspecific hybridization experiments there are a number of factors influencing the results. In the present case, some of the more important issues were: 1) Time of flowering could not be programmed, so only material flowering at a given time could be included; 2) The chance or risk for hitting or missing the correct phase of female and male flower development. More than 48 experiments were not successful probably due to mismatch of flower developmental phases; 3) The challenges of crossing more distantly-related species (28 miscarriage cases); 4) The challenges of including triploids (10 cases) or pollen sterile hybrids (13 cases); 5) Inappropriate timing and handling of seeds, i.e. maturation, sowing, and growing conditions; 6) Attacks of fungal, bacteria, insects and other hazards.

Our studies of hybrids and hybridization experiments were initiated on the Sri Lankan *Cryptocoryne,* so the number of successful crossings is expected to be negatively influenced by lack of general knowledge and experience.

RESULTS

The artificial hybrid combinations, CyCy plants

Within the six diploid 2*n* = 28 species (Table 1) a total of 15 combinations are theoretically possible (6 × 6 = 36, minus 6 self's => 30:2 = 15), when excluding reciprocal crosses. Of these it has been possible to obtain 12 combinations (**Figure 2)**.

A total of 264 crosses were performed within the Sri Lankan 2*n* = 28 group, 37 of which resulted in fruit formation and 17 in viable and flowering hybrid plants (Table 3). Each crossing was recorded as a CyCy number.

Of the total of 264 crossings, 10 were triploid combinations, 13 were combinations including the sterile *C.* ×*willisii*, and 28 included distantly related species. In the remaining 213 crossings, fruiting was obtained in 36 cases (c. 17%), and in 17 cases (c. 8%) viable hybrids.

Thirteen "primary" hybrids (F_1), and four secondary hybrids (F_2) were obtained.

Comments on the CyCy hybrid combinations

("*C. beckettii* × *C. walkeri*") × ("*C. beckettii* × *C. walkeri*"): CyCy 7 (Figure 3)

[NJ 23–7, Halloluwa × NJ 23–7, Halloluwa]

The naturally occurring parent NJ 23–7, has a spathe limb colouring not matching any of the recognized species and was assumed to be a hybrid between *C. beckettii* and *C. walkeri*. The hybrid CyCy 7, being a "self" segregated into spathe limb colour variants of presumed parents and NH 23–7. Our conclusion is that NJ 23–7 is a naturally-occurring fertile hybrid between *C. beckettii* and *C. walkeri*, and hence the CyCy 7 plants to be regarded as "F_2-generation" hybrids.

NJ 23–7 was used successfully in another attempt (CyCy 84) with *C. parva* and the "F_2-generation" segregated as expected.

The 18 hybrid progeny showed segregation in spathe limb colouring of the NJ 23–7 parental types, *C. beckettii* and *C. walkeri* and intermediate types (**Figure 3**). The segregation in the CyCy 84 progeny supports the hypothesis that NJ 23–7 is of hybrid origin, the parents being *C. beckettii* and *C. walkeri*.

C. beckettii × *C. parva*: CyCy 81 (Figure 4)

[NJ 23–9, Halloluwa × NJ 3094, 'Tropica', cultivated]

The 9 hybrid progeny are intermediate in size between the larger *C. beckettii* and the small *C. parva*. The spathe limb surface is somewhat rough (*C. parva* characteristics) and the limb colours vary from light reddish to yellowish while the throat and collar are black-purple. The segregation in the spathe limb colour suggests that the NJ 23–9 *C. "beckettii"* parent may be of hybrid origin.

The CyCy 81sterile hybrids corresponds with the naturally-occurring *C.* ×*willisii*, which was abundant at the Halloluwa location (See also Jacobsen, 1981a & b; Jacobsen et al., 2016).

C. beckettii × *C. parva*: CyCy 82 [NJ 23–9, Halloluwa × NJ 3094, 'Tropica', cultivated]. A second cross with the same parents as CyCy 81 (progeny 5).

(*C. beckettii* × *C. walkeri*) × *C. parva*: CyCy 84 (Figure 5)

[NJ 23–7, Halloluwa × NJ 3094, 'Tropica' cultivated]

The 21 hybrid progeny showed segregation in spathe limb colouring. With the female parent being a supposed hybrid and *C. parva* the male parent the combination may be seen as a Mendelian

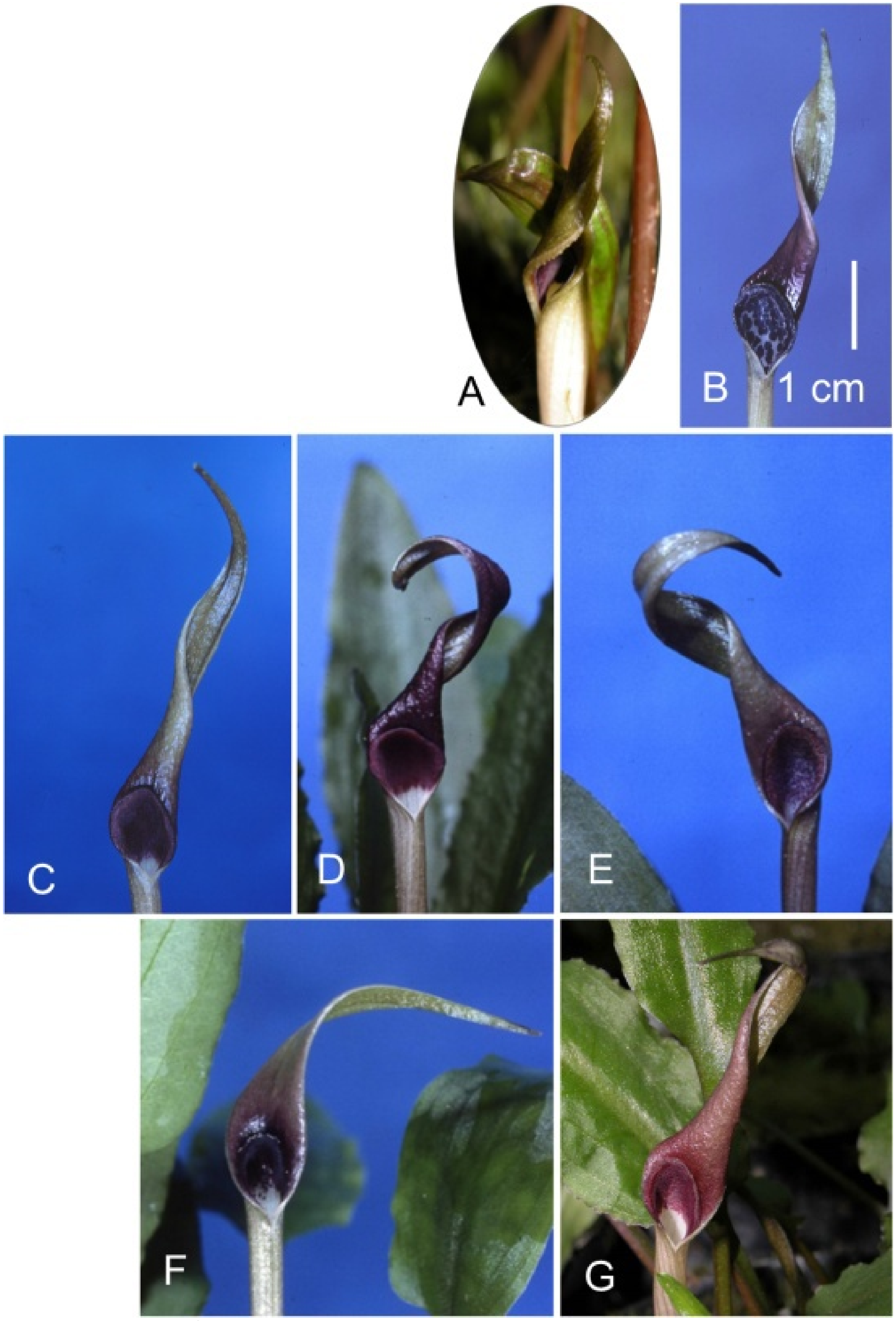

Figure 6. CyCy 121 cross. **A ♀.** KEN 334.70–03260, cult. A is a typical *C. wendtii,* as a photo of the actual accession was not available; **B ♂.** NJ 3093, Baticaloa; **C – G.** Hybrid siblings representing CyCy 121: C. *wendtii* × C. *nevillii.*

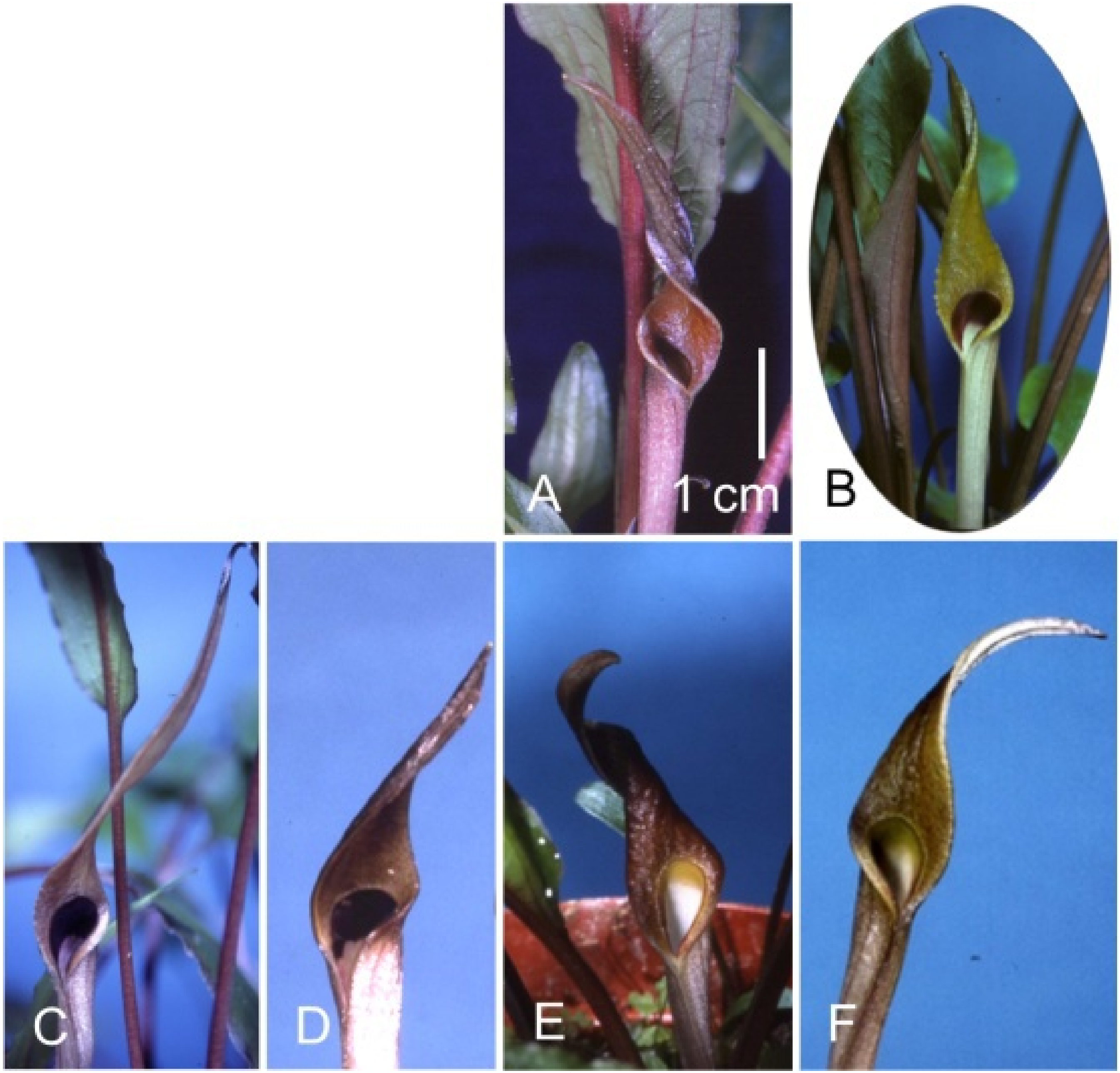

Figure 7. CyCy 136 cross. **A ♀.** P 1961/542, cult.; **B ♂.** KEN 334–70–03263, cult. B is a typical *C. beckettii,* as a photo of the actual accession was not available; **C – F.** Hybrid siblings representing CyCy 136: C. *undulata* × C. *beckettii.*

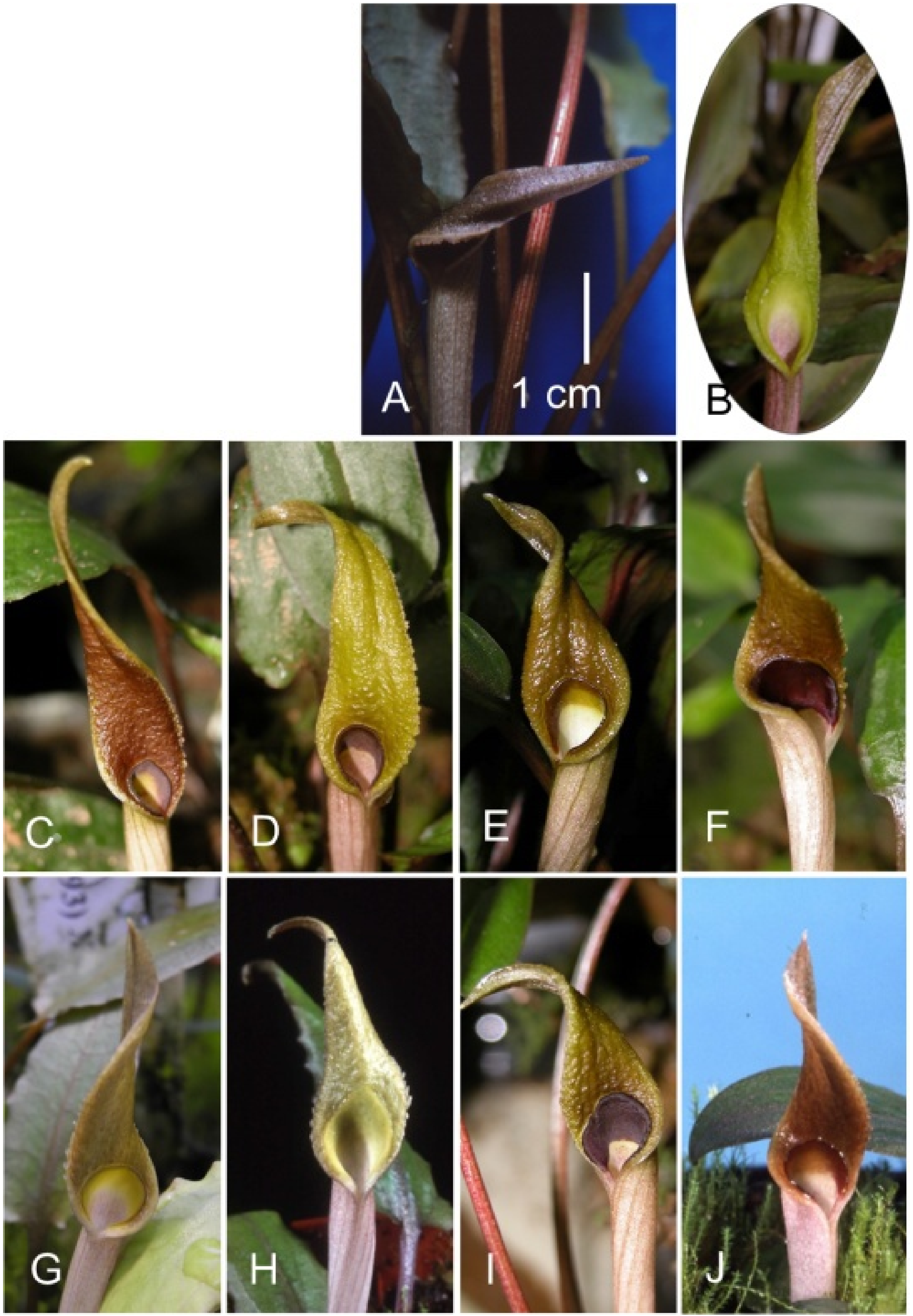

Figure 8: CyCy 144 cross. **A ♀.** NJ 2793, cult.; **B ♂.** NJ 3182, cult. B is a typical *C. walkeri,* as a photo of the actual accession was not available; **C – J.** Hybrid siblings representing CyCy 144: *C. wendtii* × *C. walkeri.*

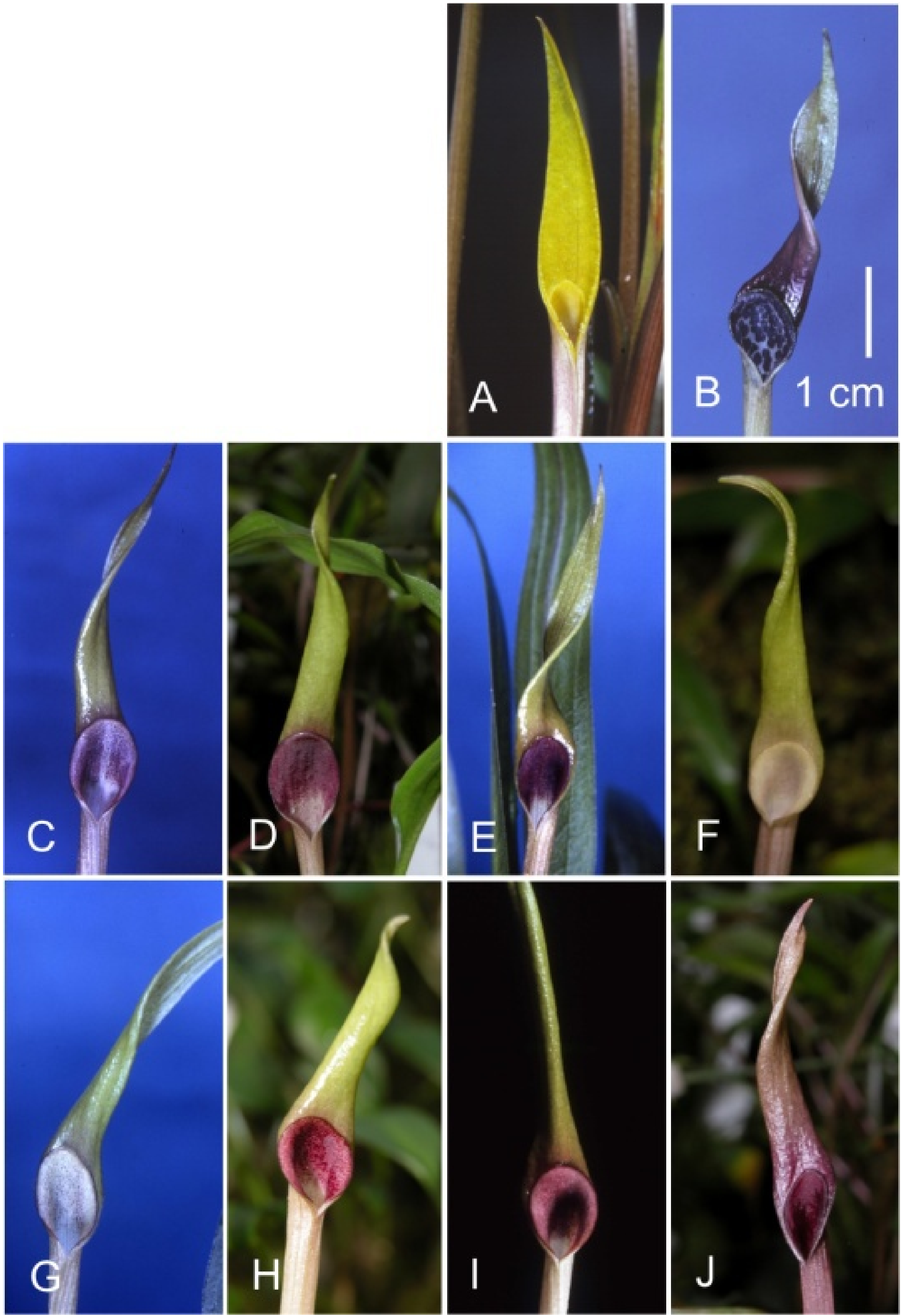

Figure 9. CyCy 158 cross. **A** ♀**.** NJ 23–6, Halloluwa; **B** ♂**.** NJ 3093, Baticaloa. **C – J.** Hybrid siblings representing CyCy 158: ["*C. walkeri* × *C. beckettii*"] × *C. nevillii.*

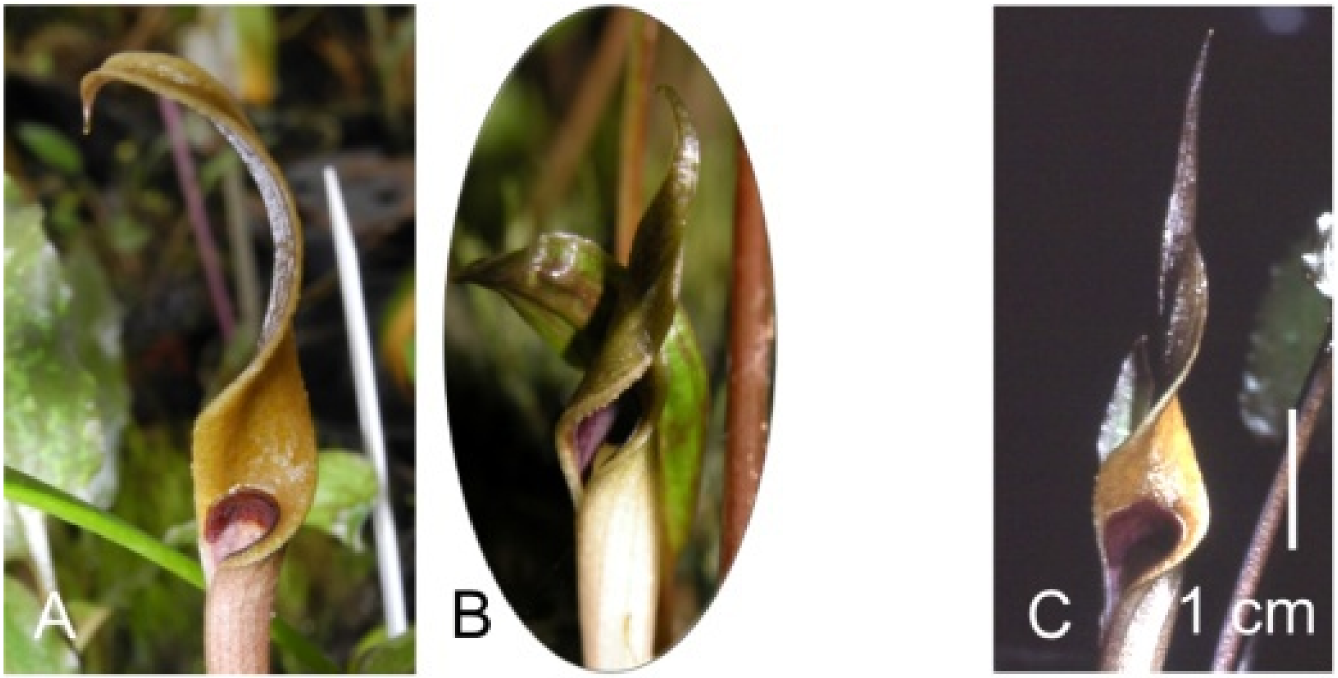

Figure 10. CyCy 184 cross. **A** ♀**.** Jayasuriya 2246, Rhuna; **B** ♂**.** NJ 3216, Malvatu Oya. B is a typical *C. wendtii,* as a photo of the actual accession was not available; **C.** Hybrid sibling representing CyCy: *C. beckettii* × *C. wendtii.*

test cross confirming that NJ 23–7 is of hybrid origin (See also Jacobsen 1981a & b, 1987; Jacobsen et al. 2016). The limb surface is slightly rugose (from *C. parva*) varying in colours from yellow to reddish; colours of the throat and collar are yellow with or without a darker rim; the throat and collar being all red-purple.

C. wendtii × *C. nevillii*: CyCy 121 (Figure 6)

[KEN 334.70–03260, cultivated × NJ 3093, Batticaloa]

The limbs of the 28 hybrid progeny were intermediate between the parents and varied somewhat in colour and shape of the spathe limb: The limb surface colour varied from black purple to reddish to yellowish and the throat and collar from black purple to yellowish with small purple spots. The leaves and the tube length resembled *C. nevillii* with long, green petioles and leaf blades.

C. undulata × *C. beckettii*: CyCy 136 (Figure 7)

[P 1961/542, cultivated × KEN 334–70–03263, cultivated]

The spathe characters of these 7 hybrid progeny segregated both in shape and colours. Some were similar to the parents, i.e. *C. undulata* having a yellowish spathe limb slightly forward twisted and a yellowish throat and collar and *C. beckettii* having a brownish spathe limb openly twisted and a black purple throat and collar. Others were intermediates with backwards bent, yellowish limb and a brownish rim on the collar.

C. wendtii × *C. walkeri*: CyCy 144 (Figure 8)

[NJ 2793, cultivated × NJ 3182, Tropica, cultivated]

The 26 hybrid progeny segregated in limb form and colours in between the parents, some having a twisted limb like *C. wendtii*, others with a more *C. walkeri*-like limb and colours.

(*C. beckettii* × *C. walkeri*) × *C. nevillii*: CyCy 158 (Figure 9)

[NJ 23–6, Halloluwa × NJ 3093, Batticaloa]

The 8 hybrid progeny showed segregation in the colours of the spathe limb from being yellow to dark purple and throat and collar opening yellow to faintly darkly spotted to conspicuously spotted. The leaves and the tube length characters bear most resemblance to *C. nevillii*.

NJ 23–6, Halloluwa, was previously referred to as *C. walkeri* due to its all yellow spathe limb. However, in CyCy 158 the "F_1"-generation segregated into various colours and intensities of the limb, bearing clear resemblance to *C. nevillii*. It is therefore suggested that the geographically more distant (Batticaloa) and morphologically more different *C. nevillii* acted as a "homozygous" crossing partner whereby CyCy 158 represents a Mendelian test cross referring NJ 23–6 *C.* "*walkeri*" to be a hybrid between *C. beckettii* and *C. walkeri*.

As control, of more than six self-pollinated *C. nevillii* (cloned individuals) did not show any segregation in the offspring.

C. beckettii × *C. wendtii*: CyCy 184 (Figure 10)

[Jayasuriya 2246, Yala × NJ 3216, Malvatu Oya]

The 3 hybrid progeny were ± intermediate between the parents.

C. undulata × *C. wendtii*: CyCy 208 (Figure 11)

[NJ 23–2, Halloluwa × NJ 2824, cultivated]

The 7 hybrid progeny showed some segregation in the colour of the spathe limb surface from red brown to light brown and in the colour of the throat and collar from red brown to light brown with a darker rim.

C. wendtii × *C. parva:* CyCy 215 (Figure 12)

[NJ 2781, cult. × 1671/16, Halloluwa]

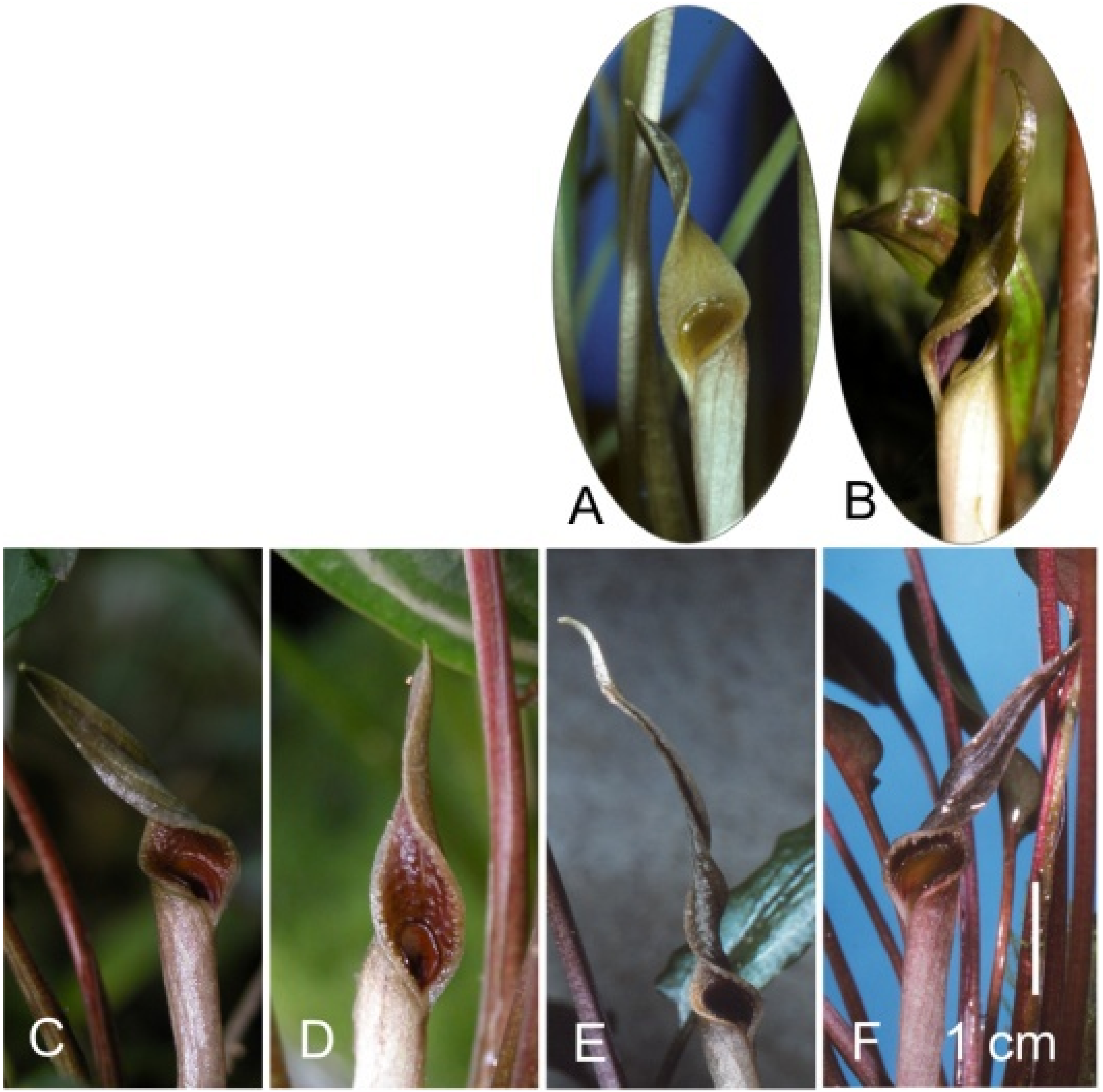

Figure 11. CyCy 208 cross. **A ♀.** NJ 23–2, Halloluwa; **B ♂.** NJ 2824, cult. A and B are typical *C. undulata* and *C. wendtii,* as photos of the actual accessions were not available; **C – F.** Hybrid siblings representing CyCy 208: *C. undulata* × *C. wendtii.*

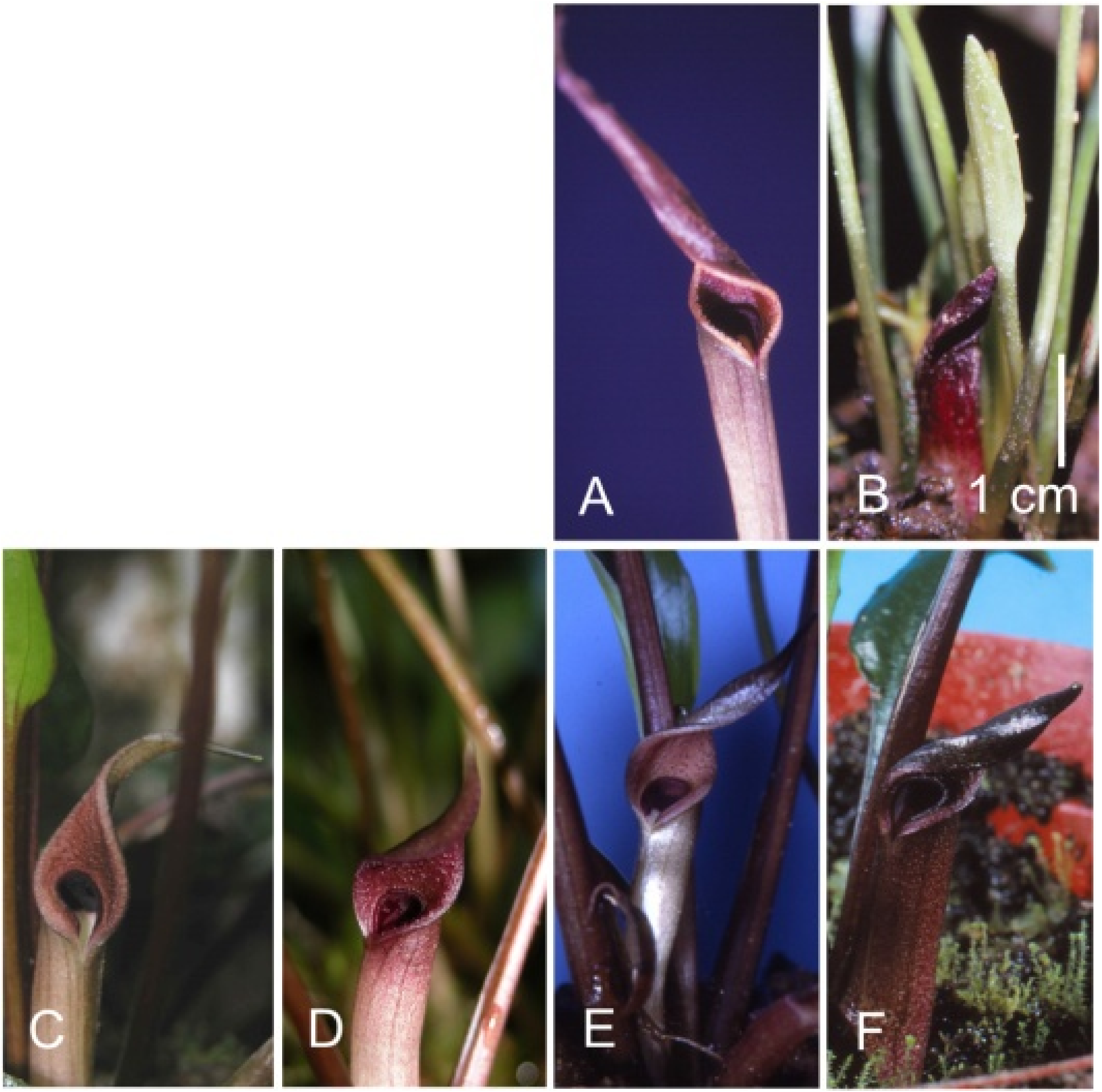

Figure 12. CyCy 215 cross. **A ♀.** NJ 2781, cult.; **B ♂.** HBH 1671/16, Halloluwa. **C – F.** Hybrid siblings representing CyCy 215: *C. wendtii* × *C. parva*.

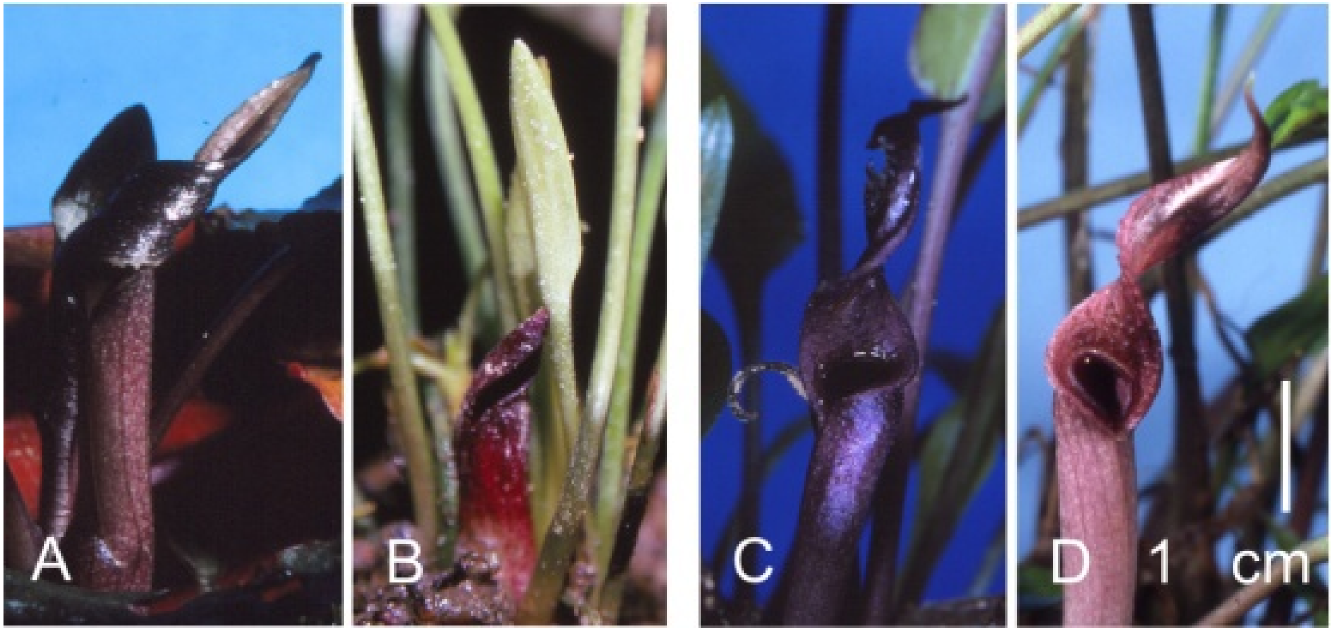

Figure 13. CyCy 216 cross. **A** ♀. NJ 3237, cult. **B** ♂. HBH 1671/16, Halloluwa; **C – D.** Hybrid siblings representing CyCy 216: *C. wendtii* × *C. parva.*

The 18 hybrid progeny were intermediate between the parents with a somewhat rugose limb (*C. parva* character), a blackish purple throat and collar (both parents) and a long-pointed limb (*C. wendtii* character).

C. wendtii × C. parva: CyCy 216 (Figure 13)

[NJ 3237, 'Tropica', cult. × 1671/16, Halloluwa]

Three hybrid progeny. This was the same combination as CyCy 215 however including a different accession of *C. wendtii*.

C. *walkeri* × C. *undulata*: CyCy 262 (Figure 14)

[NJ 2836, cultivated × P 1961/542, cultivated]

The 4 hybrid progeny were ± intermediate with slight variation in spathe limb twists and throat and collar colours varying between yellow and black purple suggesting that the *C. walkeri* accession may be of hybrid origin.

***C. nevillii* × *C.* "*walkeri*": CyCy 772 (Figure 15)**

NJ 3234, Manresa Mission × AdG 288, Halloluwa

The 9 progeny of this hybrid combination is assumed to be the reciprocal of CyCy 158, and segregates in a similar way indicating that AdG 288 *C.* "*walkeri*" is of hybrid origin, i.e. *C. beckettii* × *C. walkeri*; as NJ 23–6.

More complex hybrids

(*C. wendtii* × *C. walkeri*) × (*C. wendtii* × *C. walkeri*): CyCy 261 (Figure 16)

CyCy 144 × CyCy 144

[NJ 2793, cult. × NJ 3182, 'Tropica', cult.]

One hybrid plant was obtained, and the narrow, somewhat obliquely twisted spathe limb and the dark throat and collar are traits of *C. wendtii.*

(*C. wendtii* × *C. walkeri*) × (*C. wendtii* × *C. nevillii*): CyCy 438 (Figure 17)

CyCy 144–9 × CyCy 121–14

[NJ 2793, cult. × NJ 3182, 'Tropica', cult.] × [KEN 334.70–03260, cult. × NJ 3093, Batticaloa]

Only one hybrid plant survived and flowered. The spathe was openly twisted, yellowish limb and a red purple throat and collar, rather intermediate between *C. walkeri* and *C. wendtii.*

(*C. wendtii* × *C. walkeri*) × (*C. walkeri* × *C. nevillii*): CyCy 475 (Figure 18)

CyCy 144–9 × CyCy 158–7

[NJ 2793, cult. × NJ 3182, 'Tropica', cult.] × [NJ 23–6, Halloluwa × NJ 3093, Batticaloa]

The 9 progeny segregated into broad and raised collar and collar zone, long spathe and spathe limb (*C nevillii* characters) "brightly" coloured limbs and tube openings and collars with mostly *C. walkeri* characteristics: recurved limb, yellowish, narrow throat and collar (yellow), and broad throat and collar (and collar zone), spathe collar pronounced.

(*C. wendtii* × *C. walkeri*) × *C. nevillii*: CyCy 771 (Figure 19)

CyCy 144–19 × NJ 3234

[NJ 2793, cultivated × NJ 3182, 'Tropica', cultivated] × NJ 3234, Manresa Mission

This hybrid combination is much like CyCy 475 (except for an extra genetic dose of *C. walkeri*) in the 6 progeny resembled other *C. nevillii* hybrids.

DISCUSSION

Six Sri Lankan species are presented in Table 1; two of these are morphologically different from the others:

Cryptocoryne parva (**Figure 4 B**) has by far the smallest and shortest spathe (1.5–2.5 cm long). It is distributed in the central highlands around Kandy in the same region as *C. beckettii*, *C. undulata* and *C. walkeri.*

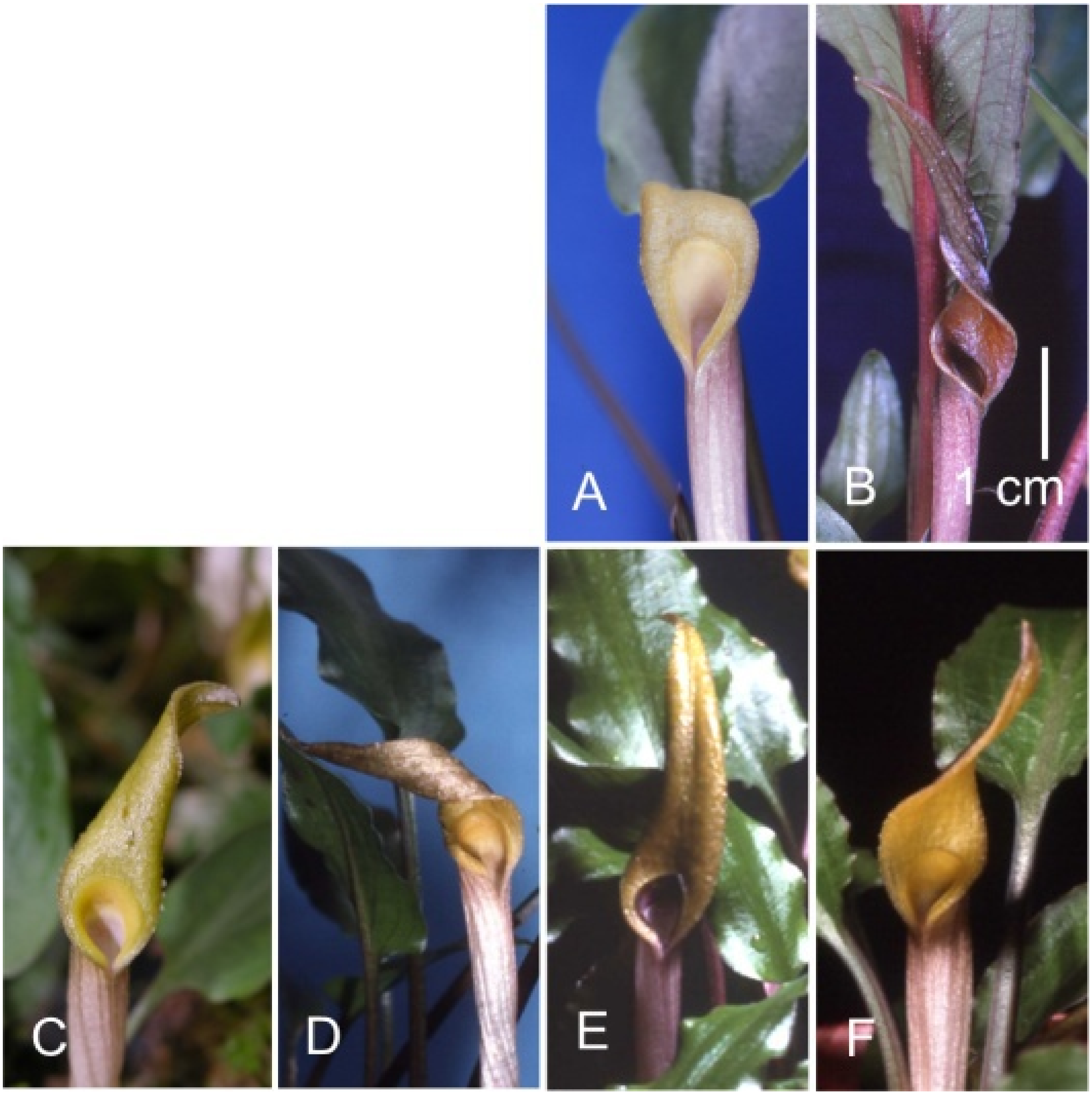

Figure 14. CyCy 262: **A ♀.** NJ 2836, cult.; **B ♂.** HBH P 1961/542, cult; **C – G.** Hybrid siblings representing CyCy 262: *C. walkeri* × *C. undulata*.

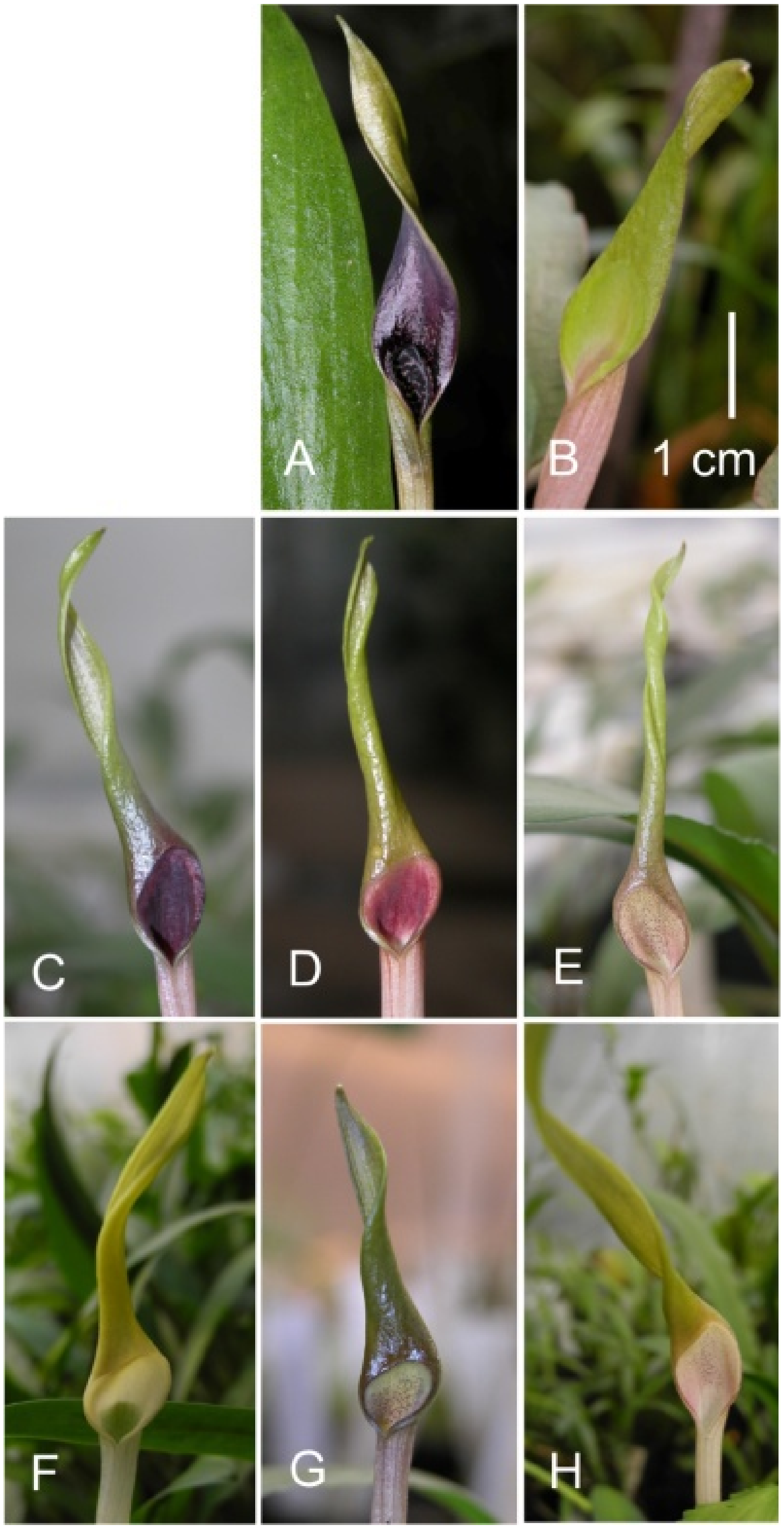

Figure 15. CyCy 772 cross: **A.** ♀ NJ 3234, Manresa Mission; **B** ♂**.** AdG 288, Halloluwa; **C – H.** Hybrid siblings representing CyCy 772: *C. nevillii* × *C. "walkeri"*.

Figure 16. CyCy 261 cross. **A ♀.** CyCy 144 [NJ 2793, cult. × NJ 3182, cult.]; **B ♂.** CyCy 144 [NJ 2793, cult. × NJ 3182, cult]; **C – D.** Hybrid siblings representing CyCy 261: [*C. wendtii* × *C. walkeri*] × [*C. wendtii* × *C. walkeri*].

Cryptocoryne parva was a crossing parent in five of the F_1-generations, viz. CyCy 81 (-82), -84, -215 (-216). However, *C. parva* was used in c. 100 crossing attempts (c. ¼ of the total attempts), but it was only successful in combinations with *C. beckettii*, "*C. beckettii* × *C. walkeri*" (NJ 23–7) and *C. wendtii*, while unsuccessful with *C. undulata* (7 attempts) and *C. nevillii* (16 attempts).

Cryptocoryne parva hybrids (*C.* ×*willisii*) have a reduced pollen fertility within the intervals 30–40 and 60–70% (Jacobsen 1977a & b). However, the fertility percentage is sufficiently high not to completely rule out the possibility that some time of function to complete successful hybridization may occur.

Cryptocoryne nevillii (**Figure 6 B**) has the longest spathe tube (up to more than 20 cm), a deviating spathe limb morphology and colours, as well as leaves with long, green leaf blades. It is found in the lowlands in the eastern coastal region.

Cryptocoryne nevillii was parent in three F_1-generations, viz. CyCy 121 including *C. wendtii*, CyCy 158 and CyCy 772, including the supposed hybrid *C. beckettii* × *C. walkeri*.

The remaining four species, *C. beckettii*, *C. undulata*, *C. walkeri* and *C. wendtii*, were successfully included as parents in all 6 possible cross combinations. Three of the species are found growing together at Halloluwa, near Kandy.

Figure 17. CyCy 438 cross. **A** ♀. CyCy 144–9 [NJ 2793, cult. × NJ 3182, cult.]; **B** ♂. CyCy 121–14 [KEN 334.70–03260, cult. × NJ 3093, Baticaloa]; **C.** Hybrid sibling representing CyCy 438: [*C. wendtii* × *C. walkeri*] × [*C. wendtii* × *C. nevillii*].

Accession NJ 23–7 was used in CyCy 7 which proved it to be of hybrid origin with *C. beckettii* and *C. walkeri* genomes as the progeny segregated morphologically. Most likely NJ 23–7 does not represent a primary F_1-generation considering that two other accessions NJ 23–6 and AdG 288, upon crossing with *C. nevillii*, (CyCy 158 and CyCy 772) resulted in offspring segregation in spathe limb colours. Since *C. nevillii* can be considered homozygous it must be the Halloluwa accessions which are responsible for the Mendelian segregation pattern, thus proving these to be of hybrid origin.

These three crossing examples revealing hidden genetic composition, suggest that many more of the Sri Lankan $2n = 28$ chromosome populations are probably of hybrid origin. And as mentioned above, self-pollinated *C. nevillii* did not show any segregation among the offspring.

The CyCy 121 *C. wendtii* × *C. nevillii* cross also presented some variation in the offspring spathe limb colours (**Figure 6 C – G**), which indicates that the *C. wendtii* accession did not come from a homogenous population.

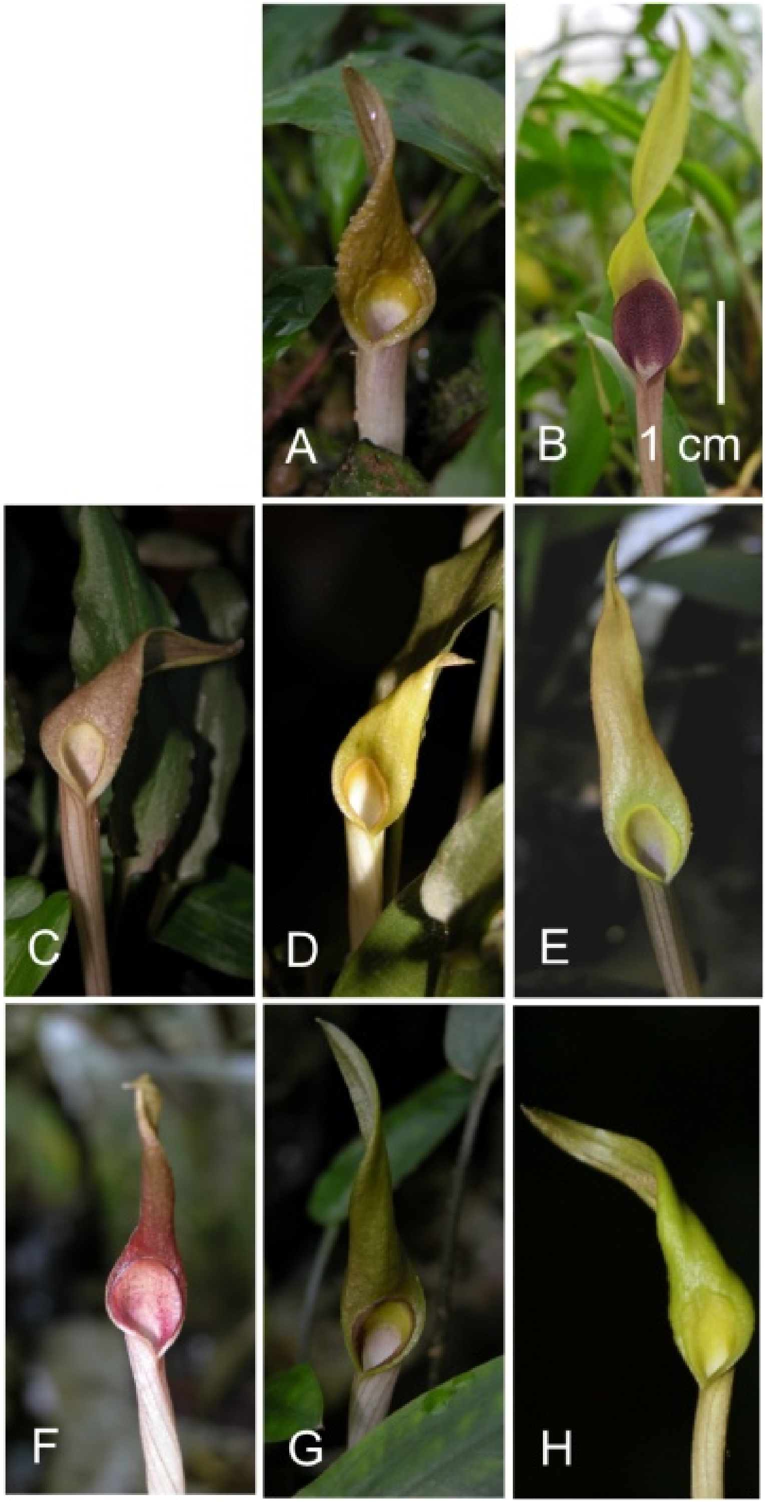

Figure 18. CyCy 475 cross. **A ♀.** CyCy 144–9 [NJ 2793, cult. × NJ 3182, cult.]; **B ♂.** CyCy 158–7 [NJ 23–6, Halloluwa × NJ 3093, Batocaloa]; **C – H.** Hybrid siblings representing CyCy 475: [*C. wendtii* × *C. walkeri*] × [*C. walkeri* × *C. nevillii*].

Figure 19. CyCy 771 cross. **A** ♀. [CyCy 144–19: NJ 2793, cult. × NJ 3182, cult.]; **B** ♂. NJ 3234, Manresa Mission; **C – D.** Hybrid siblings representing CyCy 771: [*C. wendtii* × *C. walkeri*] × *C. nevillii.*

The three hybrid combinations CyCy 136: *C. undulata* × *C. beckettii*, CyCy 144: *C. wendtii* × *C. walkeri* and CyCy 262: *C. walkeri* × *C. undulata* all show some segregation in the spathe limb colouring of the hybrid progeny, suggesting that at least one of the parents could be of hybrid origin (**Figures 7, 8 and 15**).

The more complex hybrids CyCy 438: (*C. wendtii* × *C. walkeri*) × (*C. wendtii* × *C. nevillii*), CyCy 475: (*C. wendtii* × *C. walkeri*) × (*C. walkeri* × *C. nevillii*) and CyCy 771: (*C. wendtii* × *C.* walkeri) × *C. nevillii* all involve *C. nevillii*. As with complex second-generation hybrids, the progeny will segregate, and in all three cases it is possible to see, the *C. nevillii* influence in the progeny: The leaves, the long spathe tube and the rather long, limb with a pronounced, often raised collar are *C. nevillii* characters.

The Sri Lankan *Cryptocoryne* hybrids were among the first hybrids to be investigated/produced, so it is not surprizing if lack of general knowledge about how to deal with hazards has taken a toll with respect to success.

The results indicate that crossing with triploids does not produce hybrids, at a detectable scale for us, as well as crossings with the sterile hybrid *C.* ×*willisii*.

Artificial hybrids obtained from the crossing combinations *C. beckettii* × *C. walkeri*, *C. beckettii* × *C.* ×*willisii* and *C. wendtii* × *C. walkeri*, were reported by Legro

(1963) and he speculated that *C.* ×*willisii*, could be of hybrid origin.

Hybrids obtained from crossings between Sri Lanka species: *C. beckettii* (2*x*) × *C. beckettii* (said to be 3*x*, as *C. petchii* Alston), *C. walkeri* (2*x*) × *C. walkeri* (said to be 3*x*, as *C. legroi* De Wit), and *C. undulata* × *C. wendtii*, were reported by Rataj (1975), but no further results were published.

Bednii (1998), reported that he obtained hybrids crossing *C. walkeri* (supposedly *C. legroi*, 2*n* = 3*x* = 42) and *C. usteriana* (2*n* = 34). We are in possession of these hybrids and they look mostly like *C. walkeri*, but have a more robust structure/stature, and a chromosome number of 2*n* = c. 28.

Previous work shows the wide occurrence of hybrids within the genus *Cryptocoryne* (Jacobsen et al. 2016; Jacobsen & Ørgaard, 2019). AFLP data on the *C. crispatula* Engl. complex shows that geographical closeness was reflected in genetic similarities, rather than on morphological characteristics (Jacobsen et al. 2015), and that hybridization was widespread, a feature also shown in the *C. crispatula* complex around Chiang Khan, northern Thailand (Idei et al. 2017).

Hybrids combining species across taxonomical borders gave been attempted quite widely, for example in *Hordeum*. In a study of *Hordeum* (Poaceae) a 22% success rate in obtaining viable offspring in diploid hybrids was achieved, but in this case flowering control was not a problem, and in order to obtain as many hybrid offspring as possible, embryo rescue was applied, to save the otherwise viable embryos in cases where endosperm formation was defective (Bothmer & Jacobsen 1986). These methods had been developed by researchers performing interspecific hybridization experiments in order to try to transfer valuable genetic traits from wild species of *Hordeum* to cultivated barley, *Hordeum vulgare* L. In conclusion, the *Cryptocoryne* and *Hordeum* interspecific crossing results may not be so different if all inaccuracies and hazards are taken into consideration, but the two cannot be compared more accurately, because the results of the crossings have been scored in different ways. Relatively, the success rate in possible *Cryptocoryne* crossing combinations were successful in 12 of the 15 possible combinations (80%). Similarly, the diploid crosses in *Hordeum* had a success rate of 52% of the attempted crossing combinations, and only a 22% success rate in the number of crosses including tetra- and hexaploid *Hordeum*. But the figures are not quite comparable, as the number of species crossed was higher in *Hordeum* (21 species) compared to 6 species in *Cryptocoryne*, i.e. the number of crossing attempts pr. species was higher in *Cryptocoryne*: 213 attempts including 6 species.

CONCLUSIONS

Triploid Sri Lankan *Cryptocoryne* did not produce hybrids in our crossings, nor did crossings with the sterile hybrid *C.* ×*willisii*.

It is demonstrated that the Sri Lankan species of *Cryptocoryne* of the 2*n* = 28 group are able to hybridize in nature given the opportunity. The artificial hybrids obtained in this study provide an insight into the possible combinations.

The natural occurrence of 2*n* = 3*x* = 42 chromosome plants indicates the capacity of chromosome rearrangements, however the triploids being mostly sterile (Jacobsen 1977a & b). Yet, it is generally known that both interspecific hybrid plants, and triploids produce an increased frequency of unreduced gametes, (Ørgaard et al. 1995), and the occurrence of triploids in *Cryptocoryne* could likewise be offspring from interspecific hybrids. The triploids point to possible existing, not yet discovered tetraploids. Even though a number of chromosome counts have been performed in the Sri Lankan 2*n* = 28 group, one cannot say that the group has been thoroughly investigated in this respect.

The documentation of hybrid plants occurring at Halloluwa, near Kandy exemplifies hybridization and introgression events taking place repeatedly; the success of the hybrid progeny depends on the habitats and dispersal.

Our crossing experiments contribute to the discussion of whether *C. beckettii*, *C. undulata*, *C. walkeri* and *C. wendtii* constitute separate species. The hybrids including *C. parva*, show reduced pollen fertility. In our opinion, there is no doubt that *C. nevillii* represents a separate species in relation to *C. beckettii*, *C. undulata*, *C. walkeri* and *C. wendtii*.

The present investigations support the view that "it is in the populations that evolution takes place and that they should be regarded as the naturally evolutionary centers", and that "hybridization is a driving evolutionary force which continuously produce new genotypes which subsequently are dispersed randomly in the river systems" (Jacobsen et al. 2015).

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