

Hybrids and the Flora of Thailand revisited: Hybridization in the South-East Asian genus *Cryptocoryne* (Araceae)

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ABSTRACT

The South-East Asian genus *Cryptocoryne* has been shown to hybridize more frequently than expected. Data are presented on the known naturally occurring hybrids including information on their discovery, naming, and recognition as hybrids. Many artificial hybrids produced over the years indicate that there are only relatively few barriers to crossing. A heterosis effect is prevalent in many encountered *Cryptocoryne* hybrids. Vegetative propagation is clearly an advantage in the establishment of hybrid populations and also in detecting the hybrids, since inferior hybrids have disappeared naturally. Our results on *Cryptocoryne* also suggest that when the South-East Asian floras become as well-known as temperate ones, the number of natural hybrids will be similar to those presently known from temperate regions.

KEYWORDS: Artificial hybrids, heterosis, Thai Flora, tropical natural hybrids, vegetative propagation.

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INTRODUCTION

The lack of interspecific hybrids reported in the Flora of Thailand (and the Malesian region) has been the subject of a recent work by Parnell *et al.* (2013); the topic was discussed and the general occurrence of hybrids in temperate parts of the world was also dealt with.

When comparing flora of Thailand and floristic treatments of other parts of the world there are a few facts which are reasonable to consider, namely (1) the number of species included in the flora in relation to (2) the present knowledge of the flora, (3) the

number of years the flora has been studied, and (4) the number of experienced botanists who have been working thoroughly on the flora. In relation to points 1 and 4, what are the ratios of number of recognized species and number of botanists working on the flora, and the number of years of which the flora has been studied?

We provide an example using the South-East Asian genus *Cryptocoryne*, which in Thailand is represented by 11 taxa including seven species, as outlined in Flora of Thailand account of this genus (Jacobsen *et al.*, 2012; Jacobsen *et al.*, 2015b):

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C. ciliata (Roxb.) Schott ($2n = 22$) is a mangrove species widespread in SE Asia (var. *latifolia* Rataj has $2n = 33$).

C. affinis Hook.f. ($2n = 34$) is a limestone species from Peninsular Malaysia just crossing the border in S Thailand.

C. cordata Griff. *s.l.* is widespread in the Malesian region with var. *siamensis* N. Jacobsen & Soochaloem ($2n = 102$) from the southern Thai Peninsula, and var. *cordata* ($2n = 34$) just crossing the border from Peninsular Malaysia.

C. loeiensis J.D. Bastmeijer, T. Idei & N. Jacobsen ($2n = 36$) is a recently described species from Chiang Khan.

C. mekongensis T. Idei, J.D. Bastmeijer & N. Jacobsen ($2n = 36$) is also a newly described species from W Thailand, southern Lao P.D.R. and Cambodia.

C. albida Parker ($2n = 36$) from the Thai Peninsula south of Ranong to south of Krabi.

C. crispatula Engl. *s.l.* ($2n = 36$) is a variable species and widespread in mainland SE Asia with five varieties occurring in Thailand:

var. *crispatula*

var. *balansae* (Gagnep.) N. Jacobsen

var. *flaccidifolia* N. Jacobsen

var. *kubotae* N. Jacobsen & Bastmeijer (Jacobsen *et al.*, 2015b)

var. *yunnanensis* (H. Li) H. Li & N. Jacobsen

with three additional varieties occurring outside Thailand, viz. var. *tonkinensis* (Gagnep.) N. Jacobsen, var. *planifolia* H. Zhou *et al.* and var. *decus-mekongensis* Idei *et al.*

Generally, *Cryptocoryne* species in Thailand do not coexist within river systems, except for a few cases in the peninsula. However, within the *C. crispatula* complex the situation is different as several taxa often co-occur in larger river systems such as in the Mekong catchment region, where numerous varieties are found (Idei *et al.*, 2016), as well as in other river systems in Thailand and other parts of mainland SE Asia.

MATERIALS AND METHODS

Since the middle of the 1970s we have been working with various aspects of *Cryptocoryne*: taxonomy (for an overview see Bastmeijer, 2016), morphology and pollination (Ørgaard & Jacobsen, 1998), cytology (Jacobsen, 1977a; Arends *et al.*, 1982), geographical distribution and habitat ecology via flora contributions (Ipor *et al.*, 2009; Jacobsen, 1985, 1987; Li & Jacobsen, 2010; Jacobsen *et al.*, 2012; Othman *et al.*, 2009), and molecular studies (Othman, 1997; Othman *et al.*, 2009; Ipor *et al.*, 2010; Jacobsen *et al.*, 2015a; Rosazlina *et al.*, 2015, Rosazlina 2016; Rosazlina *et al.* 2017). We have undertaken a large-scale hybridization programme, resulting in the production of more than 80 interspecific F_1 generations of which only a few have yet been published (Ipor *et al.*, 2015; Jacobsen, 1977b, 1981a, b; Jacobsen *et al.*, 2016; Rosazlina, 2016; Rosazlina *et al.* 2017).

Our insight into the various attributes of the genus has been obtained by cultivation of all described species and varieties. We have studied nearly all herbarium material deposited in the world's herbaria, as well as other recent live collections still under study, totaling more than 3000 accessions. We have also visited the habitats in most parts of the distribution area of the genus, to study the plants *in situ*.

CRYPTOCORYNE (ARACEAE)

The genus *Cryptocoryne* presently consists of 59 species together with 17 varieties, 7 named interspecific hybrids, and more than 15 unnamed hybrids. The genus is distributed in SE Asia from India, Sri Lanka and Bangladesh over southern China, Myanmar, Thailand, Lao P.D.R, Vietnam, Cambodia, Peninsular Malaysia and Sumatera to the Riau Archipelago, Borneo, the Philippines, Java, Sulawesi, Maluku, and to New Guinea (Bastmeijer, 2016).

Cryptocoryne are aquatic to amphibious herbs occurring in streams and rivers as well as in forest pools with seasonally seeping or standing water. Some of the shortest habitat streams are only a few hundred meters long (e.g., *C. elliptica* Hook.f. in Penang and Kedah in Peninsular Malaysia); others are a few km long, before running into the Indian Ocean (e.g., the habitats for *C. cordata* var. *siamensis*).

Again others are very large rivers such as the main system and the tributaries of the Mekong, where many of the varieties of *C. crispatula* occur. *Cryptocoryne ciliata* is found along coastal brackish to fresh water tidal zones throughout most of SE Asia.

Chromosome numbers within the genus vary (Jacobsen 1977a; Arends *et al.*, 1982):

2n = 36, 72; di- and tetraploids (2n = 54, triploids have also been recorded): the *C. thwaitesii* Schott group from Sri Lanka, the *C. crispatula* group, and two additional species from Borneo.

3n = 34, 68, 102; di-, tetra- and hexaploids (2n = 51 and 85, triploids and pentaploids have also been recorded), with many species from the Malesian region.

2n = 33, 66/72, 88, 110, 132; various numbers but ha-, di- and tetraploids seems to be one line with 2n = 33, 66, 132 (*C. spiralis* Retz. s.l.).

2n = 30: *C. longicauda* Engl. from the Riau region.

2n = 28 (2n = 42; triploids have also been recorded): *C. beckettii* Trim. and five additional species from Sri Lanka.

2n = 22 (2n = 33; triploids have also been recorded): *C. ciliata*.

2n = 20: *C. striolata* Engl. and an additional three species from Borneo.

A timeline for when the various taxa in *Cryptocoryne* were first described and the number of taxa recognized is provided in Fig. 1.

The rise in the number of names after Engler's (1920) revision until 1971 is mainly owing to de Wit's work up to 1971, and from then on until Rataj's revision (1975). The rise from 1975 to 1983 and 1990 marks de Wit's publications over those years.

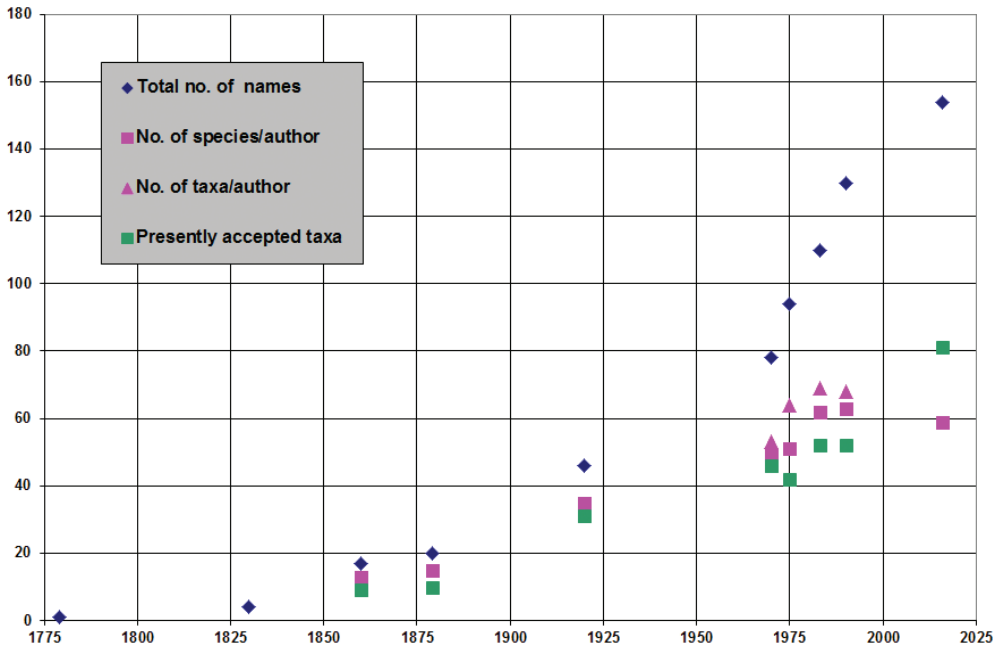


Figure 1. The species of *Cryptocoryne* described in relation to major works: Retzius (1779: *Arum spirale* Retz.), Wydler (1830: *Cryptocoryne* Wydl.), Schott (1860, Prodrumus), Engler (1879, Monographiae), Engler (1920, Pflanzenreich), de Wit (1971, Aquarienpflanzen), Rataj (1975, Revision), de Wit (1983, Aquariumpflanzen), de Wit (1990, Aquarienpflanzen) and Jacobsen *et al.* in the present work and Bastmeijer (2016). The increasing gap between the total number of names and number of taxa of the authors and presently accepted species/taxa is partly due to the rejection of older names/taxa and partly to older taxa changing status or being combined.

After 1990, the increase in names is mainly owing to the works of Bastmeijer and Jacobsen (see references here & Bastmeijer, 2016). One reason for the large number of names accumulating up to present is the transferring of taxa previously recognized as species to variety or hybrid level. New species are still being described, some are taxonomically amalgamated.

It was only after the genus began to be intensively studied in the 1960s and the following years, and after the discovery of hybrids in the 1970s (Jacobsen, 1977a, 1980, 1981a & b), that we were able to detect the hybrids. Recognizing the hybrids was only possible because of a more intensive field-work over the last 50 years. Examination of herbarium material did not provide sufficient detail to permit documentation of many hybrids, as some of the characteristics for the hybrids are rather subtle, even when studying live material. Over the last 15 years, a large amount of living material has become available and has provided insight to hybrid occurrences in *Cryptocoryne*. Even though a number of *Cryptocoryne* have been known for more than 100 years, it was not until after 1975 that it was realized that some of the plants were actually interspecific hybrids (Jacobsen, 1977a).

The number of hybrids documented has increased exponentially over the last 50 years as shown in Fig. 2 (Bastmeijer & Kiew, 2001; Ipor *et al.*, 2015; Jacobsen, 1977a & b, 1981a & b; Jacobsen *et al.*, 2002). Some of the presently unnamed hybrids will probably receive a name, while others may not (Jacobsen *et al.*, 2016 & unpubl.).

Around the turn of the millennium, many new accessions were introduced into cultivation, often helped by excellent cooperation with local amateur botanists over the internet. The awareness of hybrids within *Cryptocoryne* inspired us to check cultivated material of new accessions for possible hybrids. *Cryptocoryne* are easier and better studied living in cultivation than as dried herbarium specimens due to the fragility of the spathe, on which some of the prime distinguishing characters are found. It is almost standard procedure now for us to check new samples for pollen fertility. Many interspecific hybrids are more or less sterile, which is easily detected by assessment of the pollen fertility. The procedure is to place some pollen in a droplet of

“cotton blue” (Lactophenol Cotton Blue, e.g. Jacobsen, 1977b): fully fertile pollen stains deep blue, while less functional pollen is transparent light blue. When the practical laboratory setup is in place, the making of a pollen slide takes two minutes, adding 5–15(–30) minutes for the pollen to stain properly and then the final microscope check can be done in 30 minutes.

Hybrids between more distantly related species generally have rather low pollen fertility (stainability) and are generally pollen-sterile; hybrids between more closely related species tend to be more fertile, as in the Sri Lankan species with a chromosome number of $2n = 28$, or within the *C. crispatula* complex. Even though some hybrid plants may have a low pollen fertility, it does not necessarily mean that their ovules are also non-functional, although we have not investigated this aspect in *Cryptocoryne*. Fertility in interspecific hybrids is not an all sterile versus all fertile situation. This is well known within many cultivated plants, in e.g. *Lilium* (Tuyl & Arens, 2011) and *Brassica* (Bothmer *et al.*, 1995) where large scheme hybridization programmes have been undertaken.

Another characteristic of the hybrids is the general better growth constitution compared to the parental species owe to heterosis. Flowering frequency is also higher in the hybrids.

The number of hybrid *Cryptocoryne* accessions is relatively high compared to the total number of documented herbarium specimens. More than 3000 specimens/accessions of *Cryptocoryne* are known today, while the number of hybrid accessions amounts to more than 60, which gives a rough ratio of 50:1.

Artificial hybrids

In Copenhagen, the first *Cryptocoryne* hybrids were produced between 1978 and 1983 including mainly Sri Lankan species (with $2n = 28$). Eight F_1 generations and five complex F_2 generations were produced. Some of the hybrid combinations can be found in Jacobsen (1981a, b).

From around 2005 until 2015 a series of crossing experiments have been performed including mainly Malesian species. Altogether more than 80 interspecific F_1 generations have been obtained. It seems that there are no specific genetic barriers present within

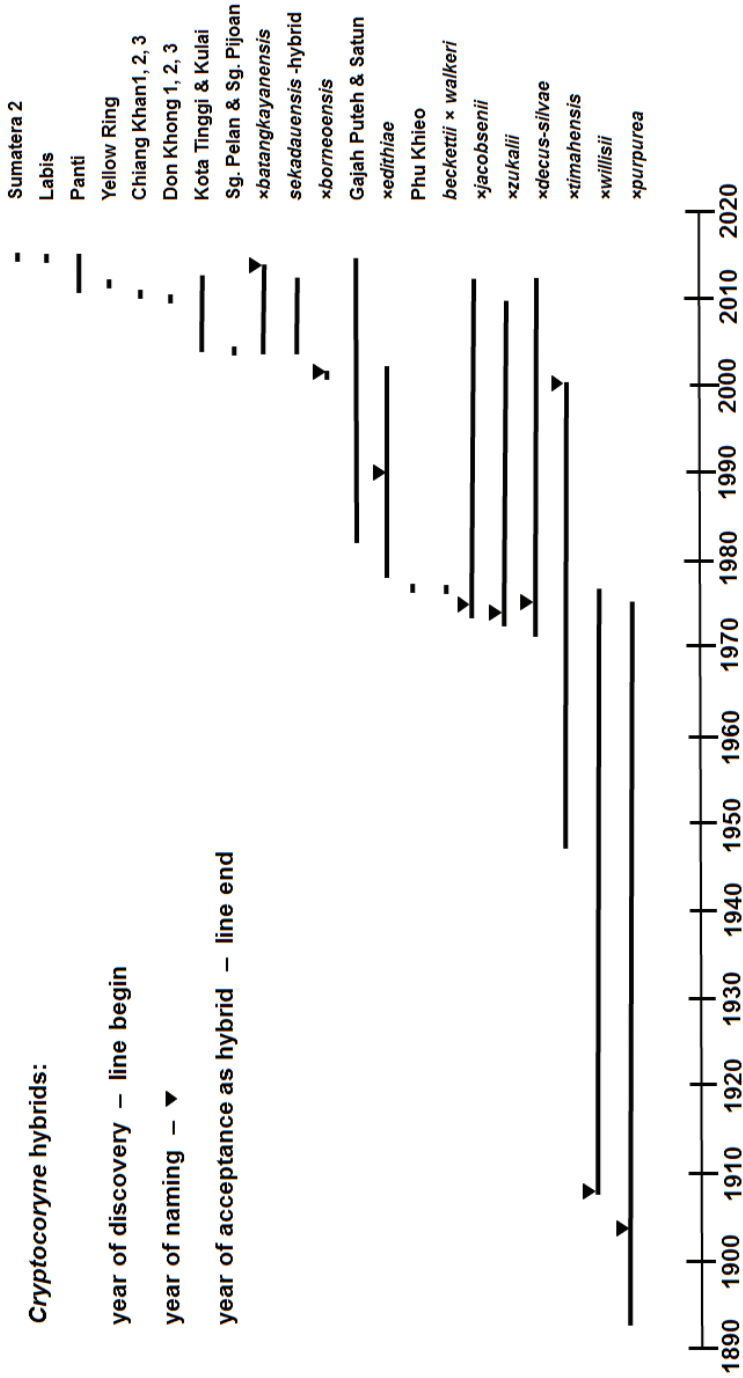


Figure 2. The year of discovery (beginning of line), the year of formal naming/description ▼ – if done – and year of recognition of hybrid status (end of line) of *Cryptocoryne* hybrids. After the initial recognition of the existence of hybrids (after 1977 & 1981), it took several years before some of the taxa were recognized. A number of hybrids have been recognized since 2000, and they are still being recognized, including two more not shown in the diagram.

the different chromosome groups (e.g. $2n = 36$, $2n = 34$, 68 , 102 and $2n = 28$). A few inter-group hybrids have also been obtained. Photos of inflorescences of a few examples of artificial interspecific hybrids are shown in Figs. 3, 4 & 5.

A primary aim of the crossing programme has been to see if it is possible to resynthesize naturally occurring hybrids and thus make it easier to detect hybrids in nature, and in new-incoming material.

The spathes of the artificial hybrids are generally morphologically intermediate between the parents. However, in combinations where one parent has twice the chromosome number of the other parent, the parent contributing the most chromosomes dominates the phenotypic appearance of the hybrid, i.e. in size, shape and colours. Seven of the hybrids that hitherto have flowered resemble naturally occurring hybrids: *C. ×willisii* Reitz, *C. ×purpurea*

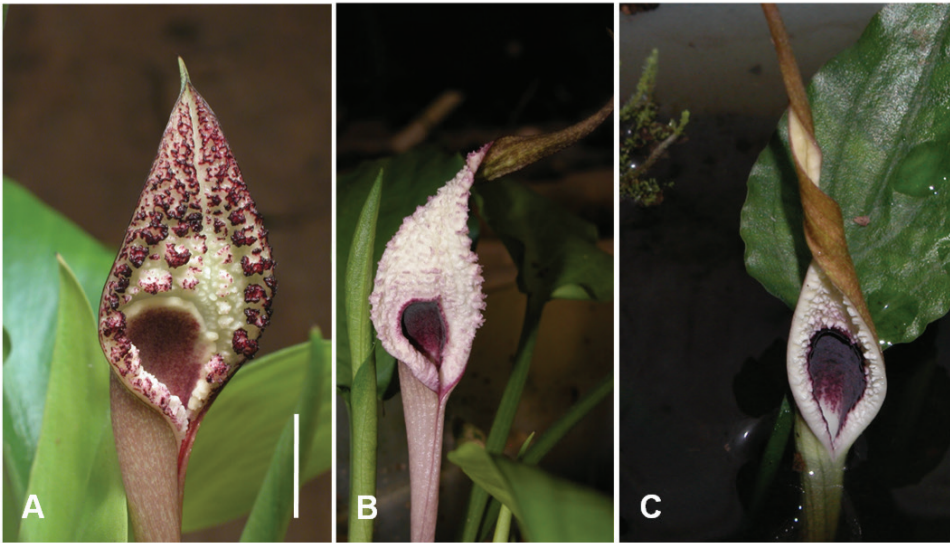


Figure 3. Inflorescences of *Cryptocoryne* parental species and their artificial hybrid: A. *C. zaidiana* Ipor & Tawan, Sarawak, $2n = 36$; B. artificial hybrid CyCy 342; C. *C. yujii* Bastmeijer, Sarawak, $2n = 34$. Photographs A by J.D. Bastmeijer, B–C by N. Jacobsen. Scale 1 cm.



Figure 4. *Cryptocoryne* parents and their artificial hybrid. A. *C. longicauda* Engl., Sarawak, $2n = 30$; B. artificial hybrid CyCy 337; C. *C. yujii* Bastmeijer, Sarawak, $2n = 34$. Photographs by N. Jacobsen. Scale 1 cm.



Figure 5. *Cryptocoryne* parents and their artificial hybrid. A. *C. crispatula* Engl. var. *balansae* (Gagnep.) N. Jacobsen, Thailand, $2n = 36$; B. artificial hybrid CyCy 489; C. *C. zaidiana* Ipor & Tawan, Sarawak, $2n = 36$. Photographs A–B by N. Jacobsen, C by J.D. Bastmeijer. Scale 1 cm.

Ridl. nothovar. *purpurea* and nothovar. *borneoensis* Jacobsen *et al.* (including *C. edithiae* De Wit), *C. ×batangkayanensis* Ipor *et al.*, *C. ×decus-silvae* De Wit (incl. *C. jacobsenii* De Wit and some of the unnamed hybrids from Kulai, Kota Tinggi, Panti, Labis and Sg. Bebar (Jacobsen *et al.*, 2016 & unpubl.), *C. ×zukalii* Rataj and *C. ×timahensis* Bastmeijer. We have produced far more interspecific hybrids, than we have found “unidentified” in nature; in a number of cases our hybrids represent combinations which we do not expect to find in nature because of disjunctive geographical distribution of their parents (e.g. Fig. 5). A present day disjunct distribution may not have been disjunct during the last glaciation, and it cannot be entirely out ruled that it is possible to find hybrids between currently disjunct species (cf. maps by Voris (2000) and Sathiamurthy & Voris (2006)).

Natural hybrids

Cryptocoryne ×*purpurea* Ridl.

The first encounter of *Cryptocoryne purpurea* is from 1892 when Ridley collected it somewhere near Kota Tinggi, Johor. It was cultivated in the Botanical Garden in Singapore, and shipped to Europe in 1898. It flowered at Kew and was pictured as *C. griffithii* Schott in Botanical Magazine in 1900 (t 7719); in 1904 Ridley described this plant as a new species: *C. purpurea*. In the years following it

was cultivated widely as an aquarium plant in Europe, although it almost disappeared towards the end of the century. As a result of the study of chromosome numbers and pollen fertility, it was suggested that *C. purpurea* was a hybrid based on the low pollen fertility (Jacobsen, 1977a). Evidence for this assumption gained support over the years and it is now generally accepted as a hybrid (Fig. 6) between the diploid *C. cordata* var. *cordata* and the similarly diploid *C. griffithii*; both parents and hybrids are found in Johor (Othman *et al.*, 2009; Rosazlina, 2016; Rosazlina *et al.* 2017).

Owing to a similar hybrid from southern Kalimantan: *C. cordata* Griff. var. *grabowskii* (Engl.) N. Jacobsen × *C. griffithii*, the Peninsular Malaysian hybrid is designated *C. ×purpurea* nothovar. *purpurea* and the southern Kalimantan hybrids as *C. ×purpurea* nothovar. *borneoensis* N. Jacobsen *et al.* (see below).

Cryptocoryne ×*purpurea* nothovar. *purpurea* and herbarium specimens were not recognized as hybrids, but a herbarium specimen collected by J. Sinclair No. 8356 (E), on 3rd April 1955, from Sg. Udang Forest Reserve, Melaka, Peninsular Malaysia, and named *C. cordata* Griff. demonstrates the point: Many people (including some of the present authors) have seen this specimen and agreed - or at least not disagreed - that it represented *C. cordata*. During a field trip to Melaka in 2011 we searched for the Sg.



Figure 6. *Cryptocoryne* ×*purpurea* Ridl. nothovar. *purpurea*. A. *C. griffithii* Schott, $2n = 34$; B. *C. cordata* Griff. var. *cordata*, $2n = 34$; C (& background). This interspecific hybrid nothovar. *purpurea* plant, most probably stems from the type collection by Ridley in 1892. Photographs by N. Jacobsen. Scale 1 cm.

Udang locality, found it at an allocation that is now a recreational park with *Cryptocoryne* growing in a pond with water flowing through it. In two small streams within 2 km we sampled two *Cryptocoryne* populations. Although they differed from our image of *C. ×purpurea* nothovar. *purpurea*, we referred them to nothovar. *purpurea*, as we did with the Sg. Udang pond sample after inspection of the flowering cultivated accession (Fig. 7). We evidently sampled three diverse *C. ×purpurea* nothovar. *purpurea*, differing in the shape, surface structure and colour of the spathe limb. Today we recognize these populations as a “hybrid swarm” representing three hybridization events with different parental genotypes (Rosazlina, 2016; Rosazlina *et al.* 2017). A pollen check from the original Sinclair No. 8356 herbarium specimen is expected to show that it is sterile.

Cryptocoryne ×*willisii* Reitz

Cryptocoryne willisii from Sri Lanka was described in 1908 and has been a popular aquarium plant ever since. Based on a reduced pollen fertility

Jacobsen (1977a, b) suggested, that it could be a hybrid. Later Jacobsen (1981a, b) showed that artificial crossings between *C. beckettii* Trim. and *C. walkeri* Schott (and the fertile hybrid *C. beckettii* × *C. walkeri*) with *C. parva* De Wit (all with $2n = 28$) resulted in plants similar to *C. willisii*, giving rise to the suggestion that the latter should be given hybrid status, designated *C. ×willisii* Reitz (Fig. 8). The artificial hybrid combination (CyCy 84) made between the natural hybrid *C. beckettii* × *C. walkeri* and *C. parva* showing segregation in the offspring (Fig. 9), an example of a simple Mendelian test cross.

C. ×timahensis Bastmeijer

The first records of *C. ×timahensis* (Bastmeijer & Kiew, 2001) from Bukit Timah in Singapore are from 1948 and subsequent years were labelled as *C. griffithii* Schott, until it was described as a hybrid in 2001. The chromosome number of *C. ×timahensis* was originally counted as $2n = 54$, but a recounting of the type material showed $2n = 34$. *C. ×timahensis* was originally assumed to be a hybrid between

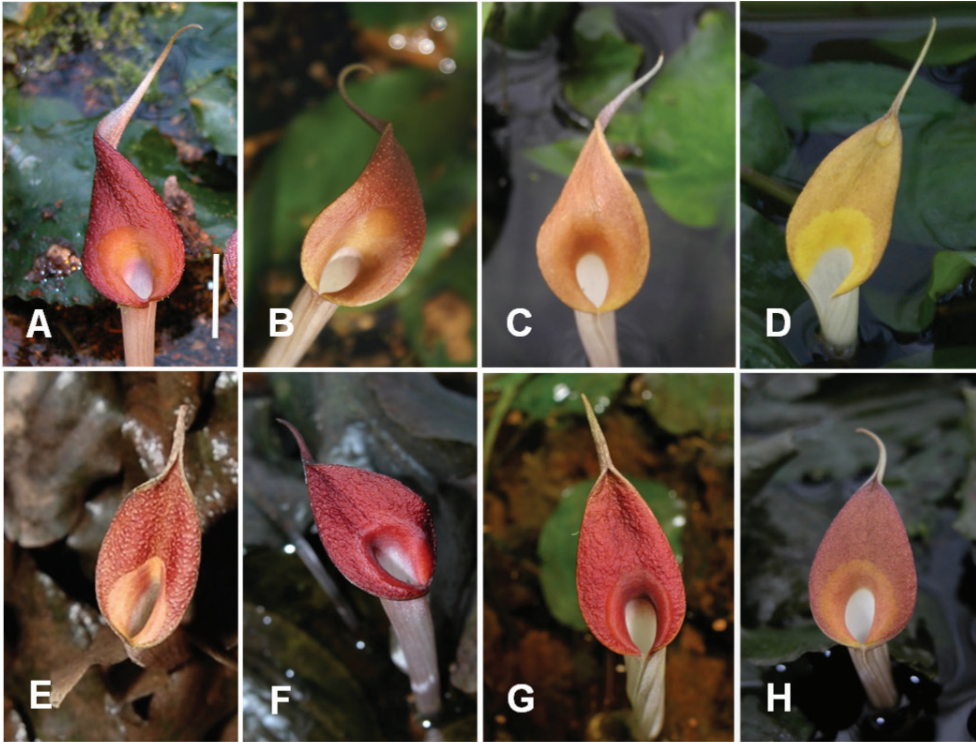


Figure 7. Spathes of different accessions of *Cryptocoryne* \times *purpurea* Ridl. nothovar. *purpurea* from southern Peninsular Malaysia: A. 1671/2, type collection; B–D. NJM 11-13, -14, -16, Sg. Udang region, Melaka; E. NJM 11-28, Sg. Sedeli Kechil, Johor; F. NJM 04-30, Sg. Likut, Johor; G. B 1311, Tasik Bera, Perak; H. NJ 3412, unknown locality, probably Johor; all $2n = 34$. Photographs E by J. Siow, G by J.D. Bastmeijer, A–D, F, H by N. Jacobsen. Scale 1 cm.

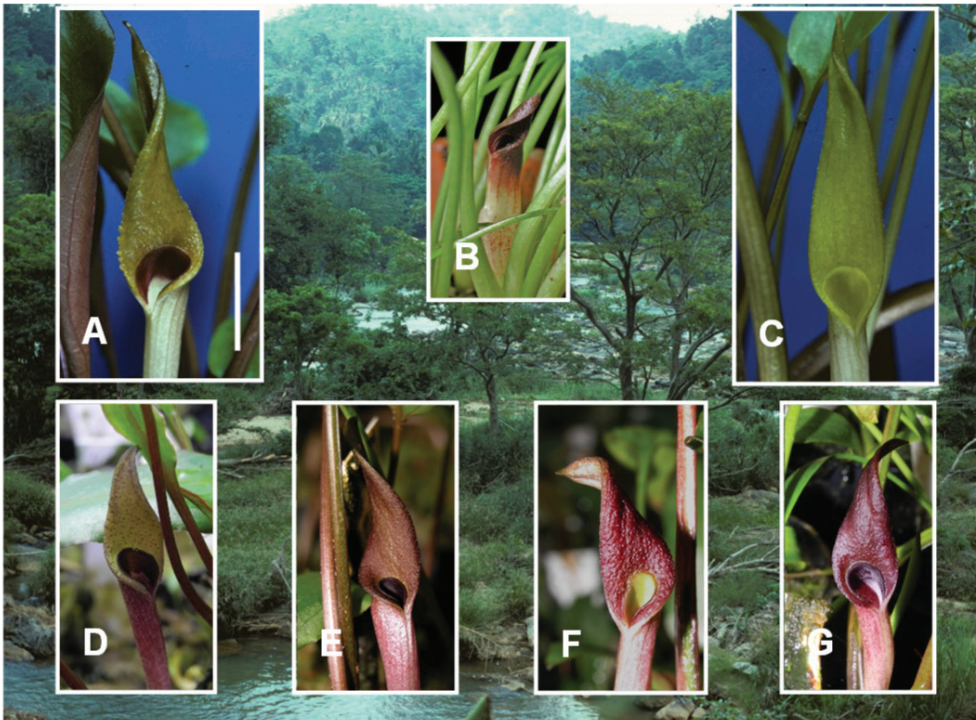


Figure 8. Different genotypes of *Cryptocoryne* \times *willisii* Reitz from Halloluwa (background), near Kandy, Sri Lanka with their parents: A. *C. beckettii* Trim.; B. *C. parva* De Wit; C. *C. walkeri* Schott; D–G: *C. x willisii*; all $2n = 28$. Photograph B by J.D. Bastmeijer, A, C–G by N. Jacobsen. Scale 1 cm.

C. cordata var. *cordata* and *C. nurii* var. *nurii*, but the artificial hybrid shown in Fig. 10 E clearly shows that it is a hybrid between *C. nurii* var. *nurii* and *C. schulzei*. A cultivated specimen from the type population of *C. ×timahensis* is shown in Fig. 10 (C & background), lined up with the parents (A & B) and their artificial hybrid E (CyCy 764: *C. nurii* Furt.

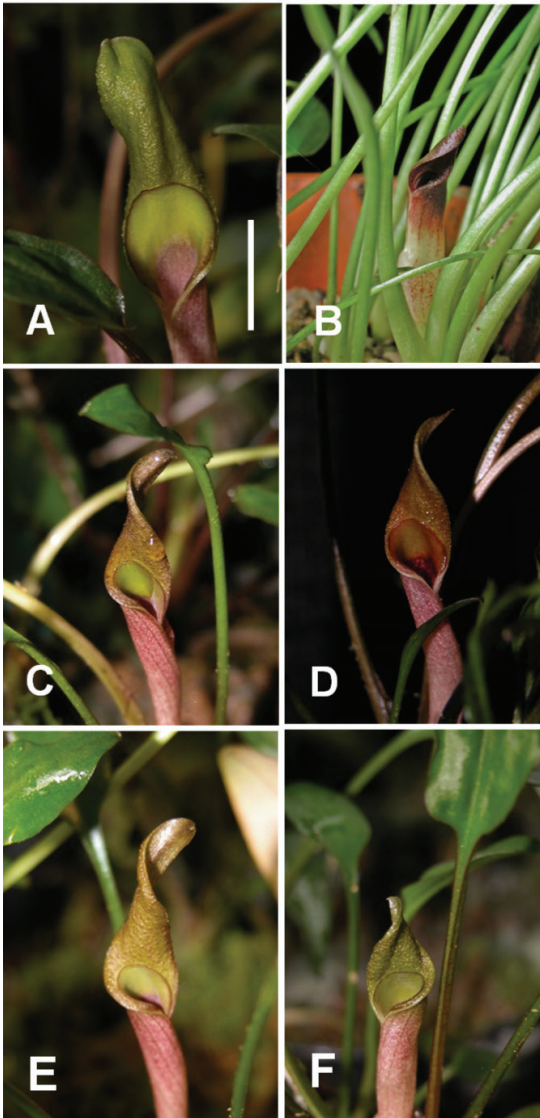


Figure 9. Artificial hybrids between the natural hybrid: A. *C. beckettii* Trim. × *C. walkeri* Schott and B. *C. parva* De Wit; C–F. showing segregation in the offspring in the artificial hybrid; all $2n = 28$. Photographs B by J.D. Bastmeijer, A, C–F by N. Jacobsen. Scale 1 cm.

var. *nurii* ($2n = 34$) × *C. schulzei* De Wit ($2n = 34$)); D is a plant found a few km from the site shown in Fig. 11 which shows a stream in Johor, SE of Mersing where *C. nurii* var. *nurii* was found in the main stream and *C. schulzei* in the small tributary to the left. No hybrids were detected at this location.

C. ×purpurea Ridl. nothovar. *borneoensis* N. Jacobsen et. al.

In 1997 T. Idei collected a *C. purpurea*-like plant at two localities in the central part of Kalimantan Tengah: Sungai Lawak and Sungei Koru (Sg. Kola), and these collections were later communicated to us by Y. Sasaki (Fig. 12). The chromosome number was $2n = 51 (= 3x)$, i.e. a triploid; the shape and size of the spathe limb indicated it to be a hybrid between *C. cordata* var. *grabowskii* ($2n = 68$) and *C. griffithii* ($2n = 34$). Both assumed parental species had been found in streams and rivers nearby. In 2002 the south Kalimantan plants were given status of hybrid variety: *C. ×purpurea* nothovar. *borneoensis*. *Cryptocoryne grabowskii* Engl. was transferred to a variety of *C. cordata* (Jacobsen, 2002), while the Peninsular Malaysia plants are automatically denominated *C. ×purpurea* nothovar. *purpurea*.

Interestingly, a number of accessions of both nothovar. *purpurea* (Fig. 7) and nothovar. *borneoensis* (Fig. 12) show clear differences in fine morphological structure and colours of the spathe limb. Therefore the hybrids must have arisen from several different hybridization events from diverse parental genotypes.

C. edithiae De Wit, Fig. 12 C–F.

Fig. 13 A–G shows two artificial hybrid combinations between *C. cordata* var. *grabowskii* and *C. griffithii* resulting in similar but distinguishable hybrids, which are due to differences in parental genotypes. Fig. 13F shows a natural hybrid resembling the artificial hybrid; these latter natural and artificial hybrids resemble what has been named *C. edithiae* De Wit.

There are more *Cryptocoryne* accessions from Peninsular Malaysia of which two have been described as species: *C. decus-silvae* (incl. *C. jacobsenii* and several unnamed populations from Kulai, Kota Tinggi, Panti, Labis, “Yellow Ring” (Reitel, 2011), Sg. Bebar shown in Fig. 14, and *C. ×zukaii*, as well

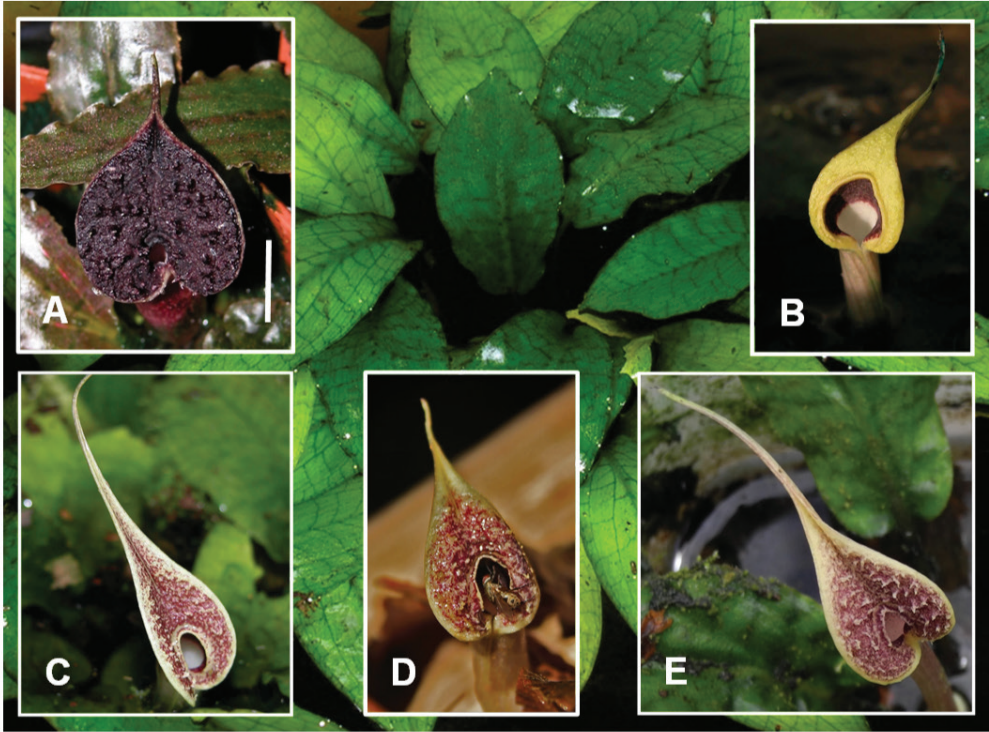


Figure 10. *Cryptocoryne* ×*timahensis* Bastmeijer from Singapore with its parents: A. *C. nurii* Furt. var. *nurii* from Johor and B. *C. schulzei* De Wit from Johor; C (& background) the natural hybrid from Singapore; D. the natural hybrid from Johor; E. the artificial hybrid: CyCy 764. Photographs: A–C, E by N. Jacobsen, D by J. Siow. Scale 1 cm.

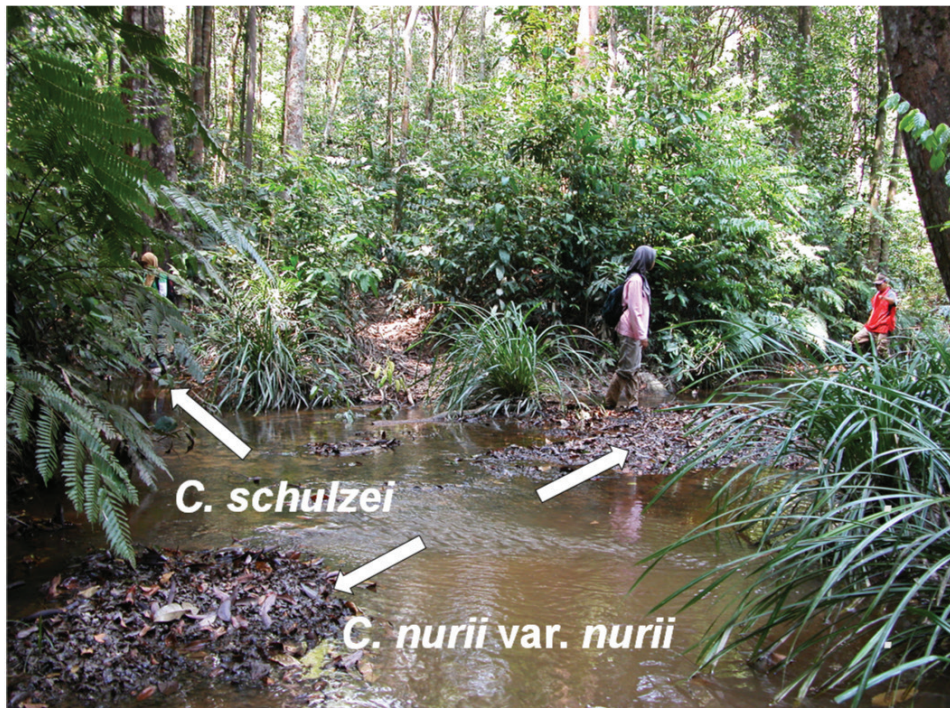


Figure 11. Stream in Johor, SE of Mersing where *C. nurii* Furt. var. *nurii* was found in the main stream and *C. schulzei* De Wit in the small tributary to the left. *C. nurii* var. *nurii* was growing submerged in large stands and emergent on sandbanks in the main stream, while *C. schulzei* was growing submerged in the small tributary. Several emergent *C. schulzei* were found growing emergent and flowering near the Cyperaceae tuft in the middle of the photo on February 15, 2001. No hybrids were observed here but they were found in another stream a few km to the east. Photograph by N. Jacobsen, March 5, 2011.

as a further number of unnamed accessions from other areas, e.g. Satun, Gajah Puteh, Sg. Pelan, Sg. Pijoan, and Sumatera No. 2 (Jacobsen *et al.*, 2016 & unpubl.). The low pollen fertility of these accessions indicates hybrid origin, as are the already accepted *C. ×timahensis*, and *C. ×batangkayanensis* Ipor *et al.* (2015).

Within the mainland Asia region, the total number of *Cryptocoryne* species is relatively low, with most of the variation encountered by now included in *C. crispatula* (8 varieties, see Bastmeijer 2016). The first hybrid was between *C. crispatula* var. *balansae* and *C. crispatula* var. *crispatula* at Phu Khieo (Jacobsen, 1980), viz. Fig. 15. Investigations

in the Mekong basin in the Lao P.D.R. at Don Khon and surroundings have shown that hybridization has taken place at least between *C. crispatula* var. *crispatula*, *C. crispatula* var. *decus-mekongensis* and *C. mekongensis*, as hybrid swarms have been found (Fig. 16). A comparable situation also occurs around Chiang Khan, Loei prov., Thailand, where numerous spathe types are found (variation in size, colours, limb coiling, and \pm fertile pollen), but the complexes here have not yet been investigated further (Idei *et al.*, 2016).

A molecular study of *Cryptocoryne crispatula* s.l. geographically occurring, among other places, in the lower part of the Sok River (below Khao Sok

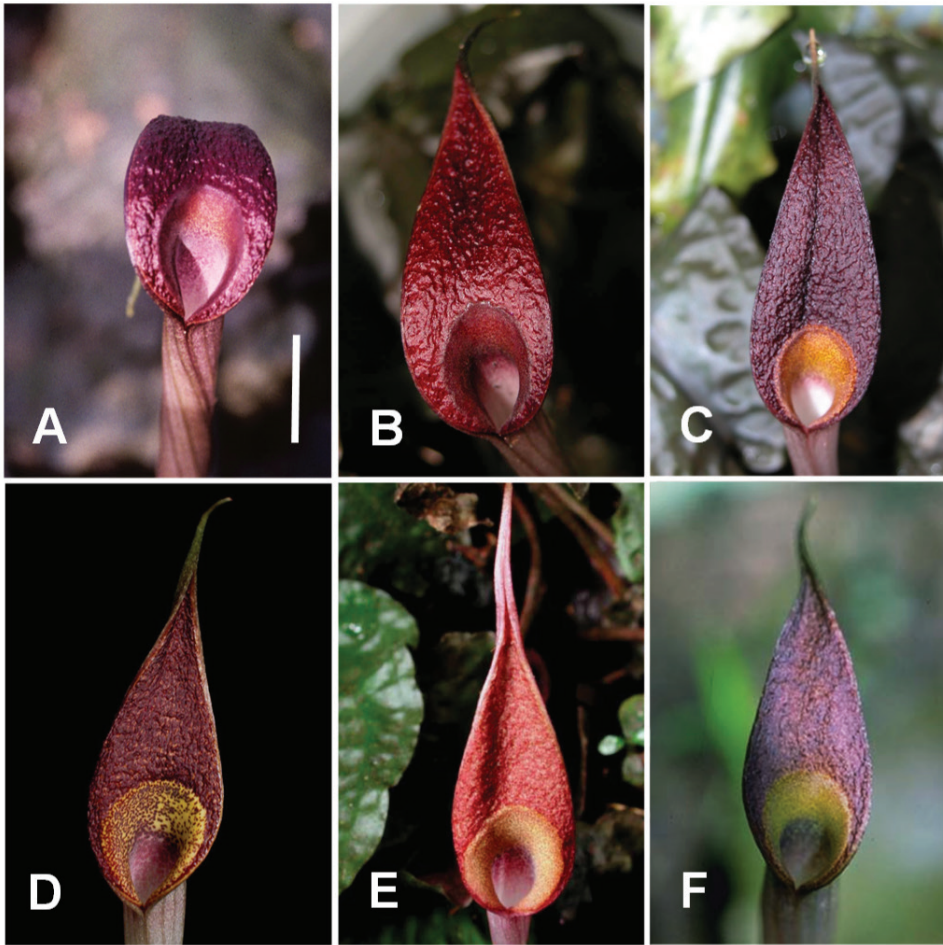


Figure 12. *Cryptocoryne ×purpurea* Ridl. nothovar. *borneoensis* Jacobsen *et al.*, $2n = 51$, showing the variation found in spathes from different accessions from southern Kalimantan: A. Sasaki SP2 (B 830), Cempaga Punbu; B. Idei B-02 (B 994), Pelantaran Hulu; C-F. showing different spathes of what has been called *C. edithiae* De Wit: C. Sasaki I-KOTT (B 960), Sg. Terantang; D. Kishi TB06-1 (B 1156), Sukamara; E. Sasaki I-SAKK1, Sg. Koru; F. M 52, from type specimen of *C. edithiae*, N of Sampit. Photographs by A–D: N. Jacobsen; E: Y. Sasaki; F: F. Möhlmann. Scale 1 cm.

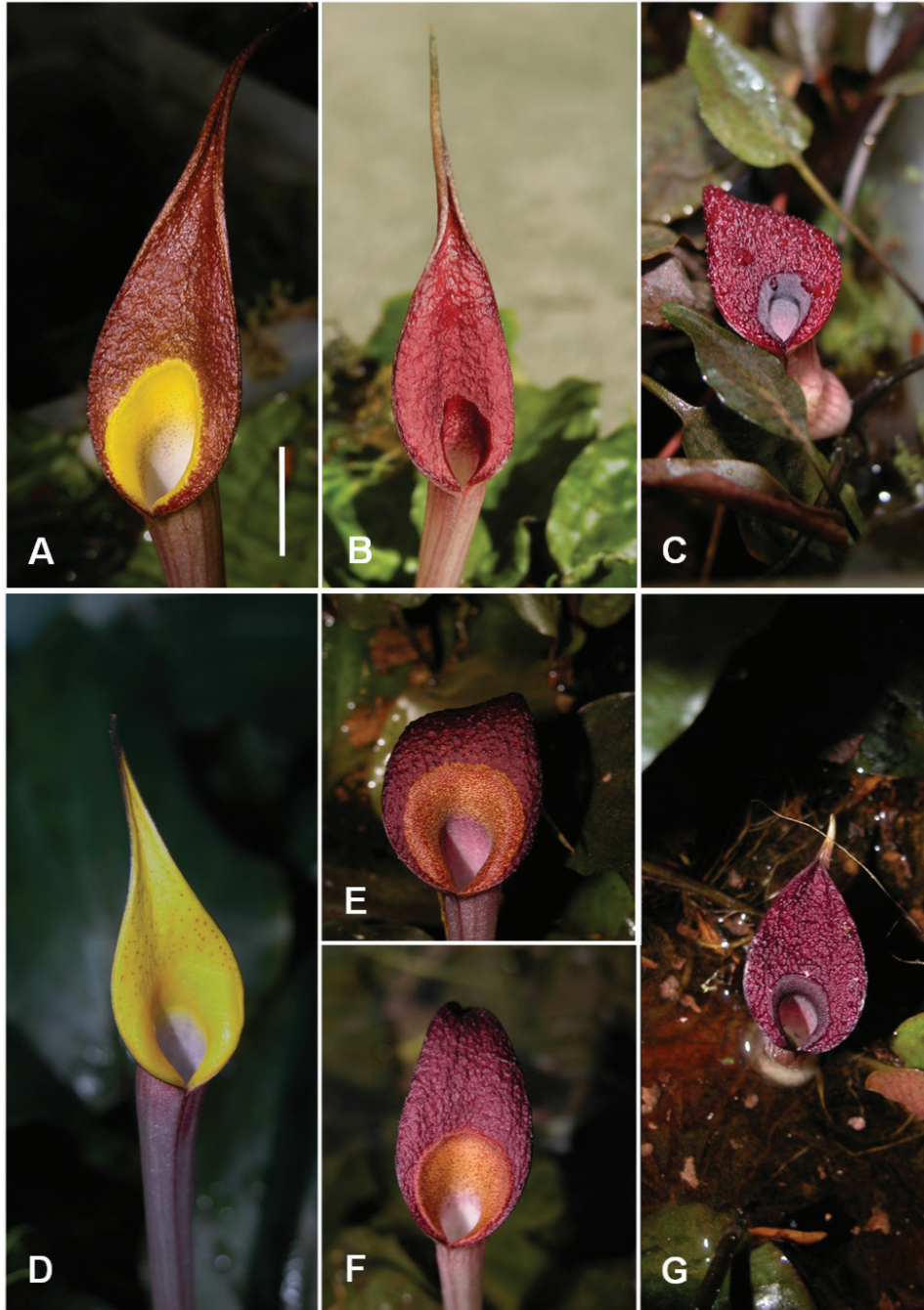


Figure 13. Artificial hybrids between *Cryptocoryne cordata* Griff. var. *grabowskii* (Engl.) N. Jacobsen and *C. griffithii* Schott: A–C: CyCy 749: A. *C. cordata* var. *grabowskii* (SW 0303 (B1025)), Desa Gunung Raja, Kalimantan, $2n = 68$); B. interspecific hybrid, $2n = 51$; C. *C. griffithii* (Idei B-04 (B 1012)), Parengaan, Kalimantan, $2n = 34$). D–G: CyCy 331: D. *C. cordata* var. *grabowskii* (Idei A-04G (B 1065)), Sg. Siong, Kalimantan, $2n = 68$); E. interspecific hybrid, $2n = 51$; F. similar natural hybrid: Sasaki I-KOTT (B 960, Sg. Terantang, Kalimantan, $2n = 51$); G. *C. griffithii* (Idei D-01A (B999)), Nanga Bulik, Kalimantan, $2n = 34$). Photographs by N. Jacobsen. Scale 1 cm.

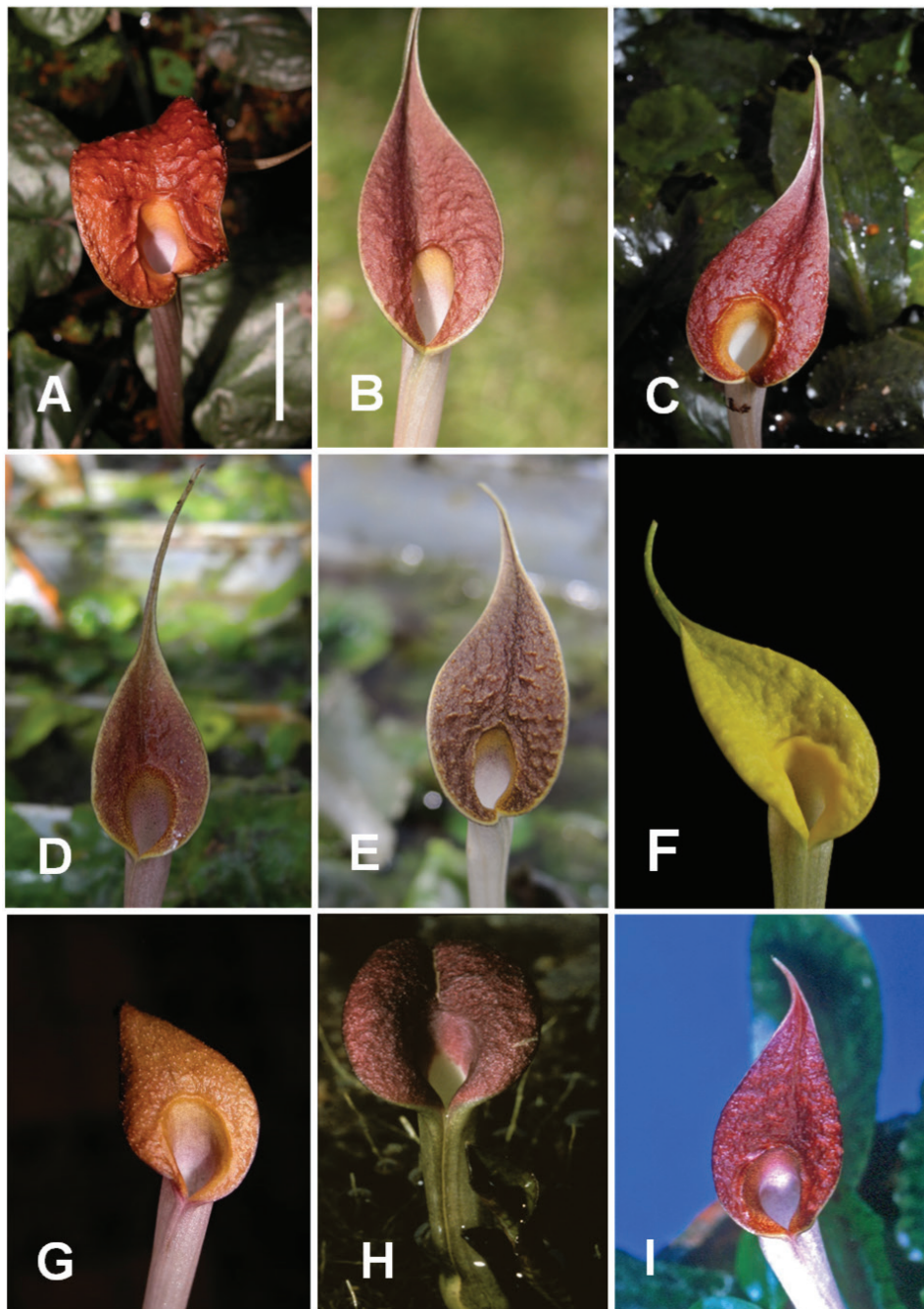


Figure 14. *Cryptocoryne* \times *decus-silvae* De Wit showing different morphology and colours of the limb of the spathes in plants from different localities: A. Kota Tinggi, Johor; B. Kulai, Johor; C. Panti B. S., Johor; D. artificial hybrid: CyCy 847 [*C. nurii* Furt. var. *nurii* \times *C. cordata* Griff. var. *cordata* (B 802, Johor \times NJT 02-21, Narathiwat, S Thailand)]; E. Labis, Johor; F. Yellow Ring, Pahang; G. Sg. Bebar, Pahang; H. type of *C. decus-silvae*, Johor; I. type of *C. jacobsenii* De Wit, probably Johor. Photographs: A–E, G, I. by N. Jacobsen, F. by J.D. Bastmeijer, H. by H. C. D. de Wit. Scale 1 cm.

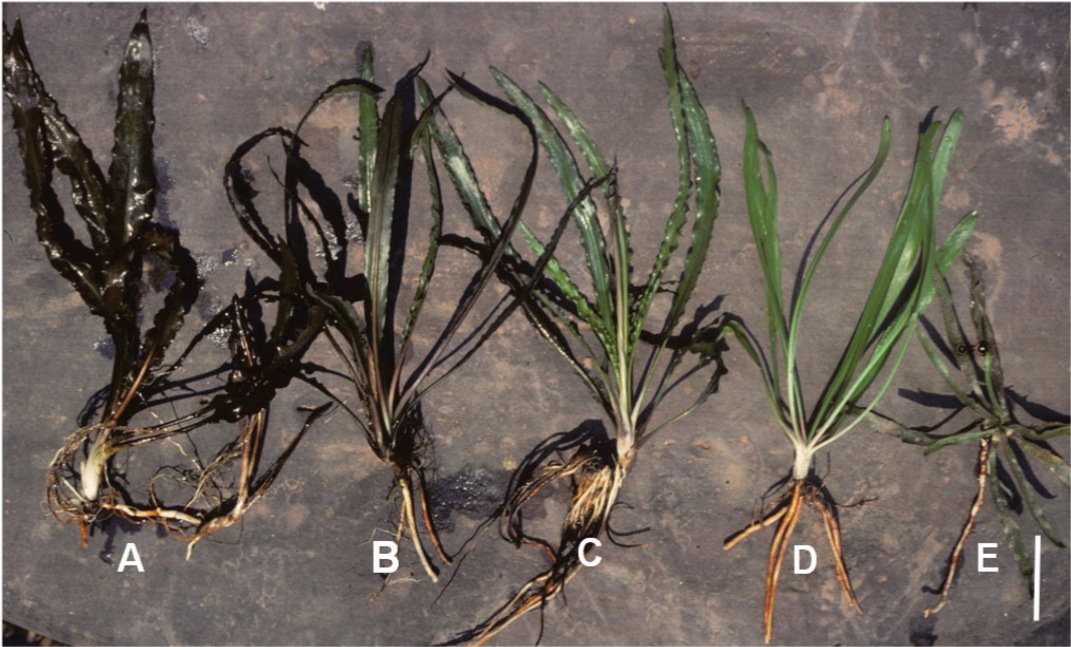


Figure 15. *Cryptocoryne crispatula* Engl. from Phu Khieo Wildlife Sanctuary, Thailand. A. NJ 77-37, submerged var. *balansae* (Gagnep.) N. Jacobsen; B. NJ 77-36, submerged intermediate type (hybrid); C. NJ 77-36, half submerged intermediate type (hybrid); D (shaded) and E (sun exposed) NJ 77-38, emergent var. *crispatula*. Photograph by N. Jacobsen. Scale 2 cm.

N.P.), showed that *C. albida*, *C. crispatula* var. *balansae* and *C. crispatula* var. *flaccidifolia* have a high degree of gene exchange and introgression (Jacobsen *et al.*, 2015a). It has not yet been possible to point out hybrid genotypes in the vast population, partly because of the few flowering specimens, partly because of limited time.

CONCLUSIONS

The genus *Cryptocoryne* has been rather intensively studied over the last 50+ years; after a number of initial taxonomical and nomenclatural problems and mistakes had been cleared up, a reasonable and mutual “understanding and agreement” as to the taxonomic circumscription of the species and varieties has been reached. The distribution and coexistence of species are relatively well known. However, species distribution and habitat types are resulting in surprises. For instance *C. ×purpurea* nothovar. *purpurea* is known from the inland fresh water swamp, Tasik Bera (Fig. 17) in the centre of Peninsular Malaysia (Jacobsen, 1986; Jacobsen & Bogner, 1987). It has recently been found in the inner fresh water tidal zones at Sg. Sedeli Kechil,

Johor, where both parental species have been found in the same river area (Figs. 18 & 19).

We conclude that wherever two species – or varieties – of *Cryptocoryne* occur near each other, there is a good possibility that hybrids will appear. One could perhaps argue that if two species occur together, and there are no chromosome number differences which might hinder hybridization, interspecific hybrids are to be expected if the area is searched thoroughly. Even if we think we are relatively knowledgeable about *Cryptocoryne* today, we must admit that only fragments of the total area of South-east Asia have yet been investigated. And compared to the 59 species known in *Cryptocoryne* more than 20 hybrid combinations have been found; which gives a ratio taxa: hybrids of about 3:1 at the specific level. This ratio easily matches the figures provided by Ellstrand *et al.* (1996) and most recently by Stace *et al.* (2015) and Preston, & Pearman (2015) for the total of the British Isles. The genus *Cryptocoryne* may include more interspecific hybrids than species.

Compared to other plant genera the search for hybrids in *Cryptocoryne* has an advantage: the ability

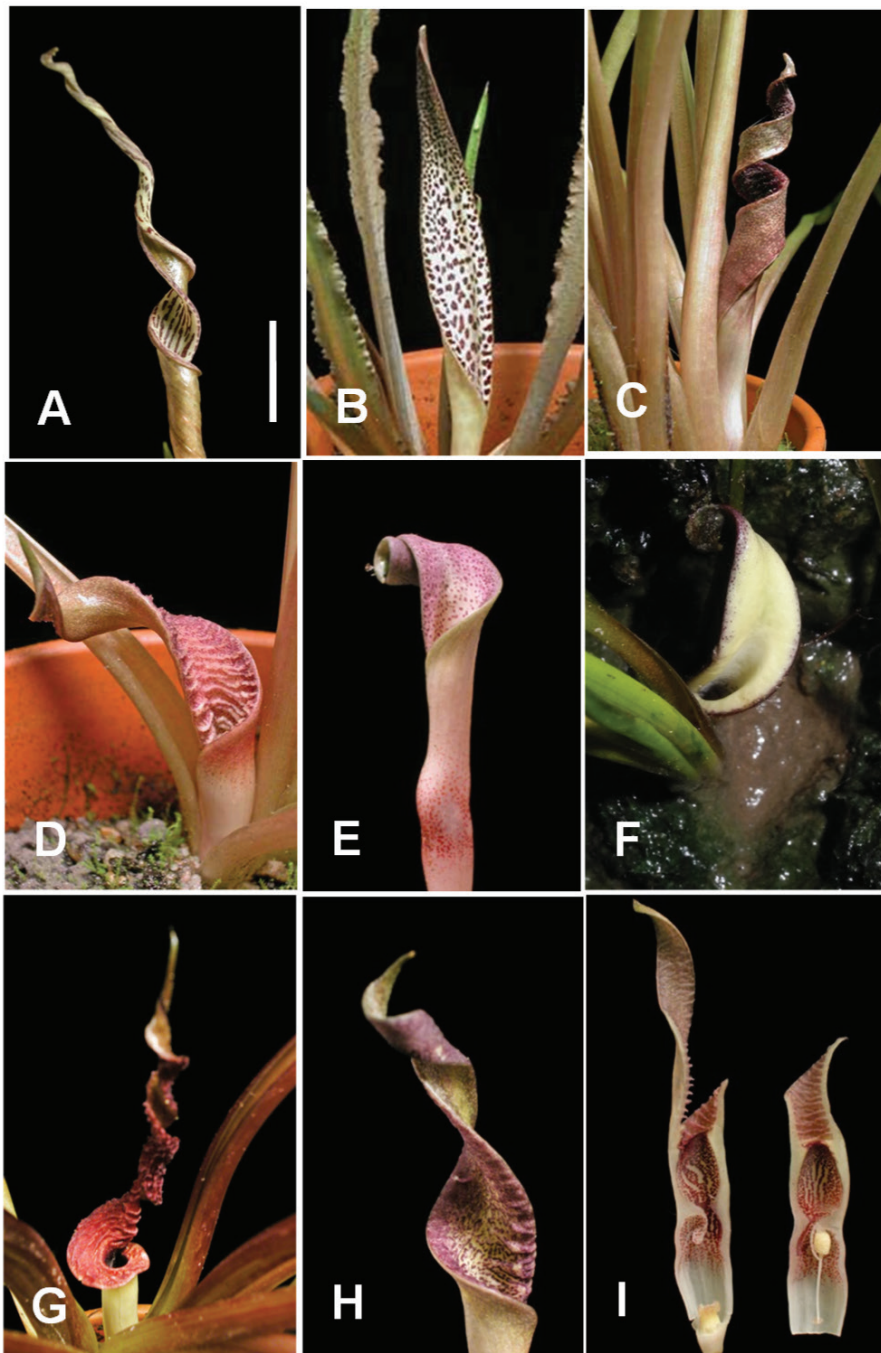


Figure 16. *Cryptocoryne* spathes from the area around Don Khon, Champasak Prov, southern Lao, P.D.R. (cultivated specimens). A. *Cryptocoryne crispata* Engl. var. *crispata* (B 1385); B. *C. crispata* var. *decus-mekongensis* T. Idei *et al.* (B 1381); C. *C. mekongensis* T. Idei *et al.* (B 1275). D. Hybrid (B 1280); E. Hybrid (B 1268); F. Hybrid (B 1380B); G. Hybrid (B 1227); H. Hybrid (B 1228); I. Hybrid (B 1229). Photographs by A–E, G–I J. D. Bastmeijer, F by N. Jacobsen. Scale 1 cm.

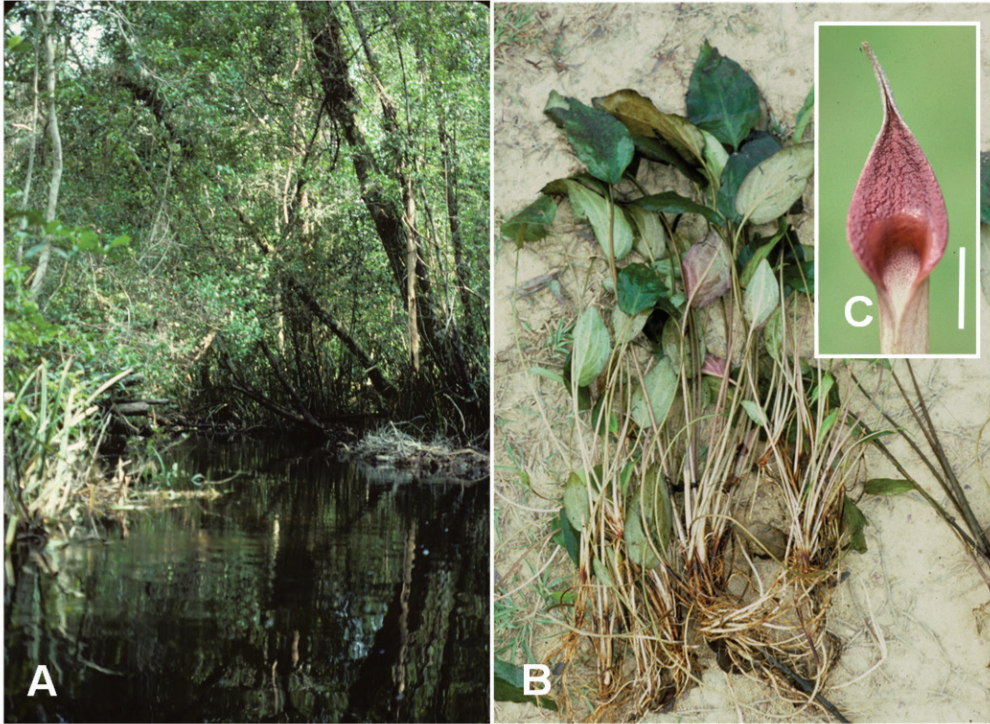


Figure 17. *Cryptocoryne* \times *purpurea* Ridl. nothovar. *purpurea*. A. showing the channels at Tasik Bera, Perak; B. plants from the channels; C. inserted the spathe limb. Photographs by N. Jacobsen. Scale 1 cm.



Figure 18. The habitat of *Cryptocoryne* \times *purpurea* Ridl. nothovar. *purpurea* at Sg. Sedeli Kechil, Johor, showing the fresh water tidal zone with mud covered tree leaves showing the high water mark (the green carpet is the South American invasive species *Mayaca fluviatilis* Aubl.). Photograph by N. Jacobsen.



Figure 19. *Cryptocoryne* \times *purpurea* Ridl. nothovar. *purpurea* at Sg. Sedeli Kechil, Johor; A. showing the mudflats at low water with the carpet of plants covering many square meters with new leaves which have not yet been covered by mud from the water; B. Spathe limb. Photographs by J. Siow. Scale 1 cm.

to propagate vegetatively by subterranean stolons, which secures that an established hybrid may propagate indefinitely and spread its genotype throughout a river system. When searching for hybrids in a *Cryptocoryne* population one is not looking at a specimen or two, but looking at whole “populations” which actually represent clones. A population of hundreds of individuals may only represent a minor part of a total population of thousands of identical clones. For the hybrid hunter this fact is of course an advantage compared to other plants which only have limited vegetative propagation, tropical trees or palms which die after a given number of years.

A prerequisite for detecting hybrids is a deep knowledge of the species, and skilled taxonomists or plant identifiers, and a “complete book of the flora” to check and to keep being informed within a given region. Is this unusual individual a hybrid? Or is it just another species with which I am not familiar?

With the present and increasing knowledge of the Thai flora and the global digital network connecting

taxonomists and knowledgeable local collectors, we agree with the prediction of Parnell *et al.* (2013) that there will be a significant rise in the numbers of hybrids being reported in the coming years.

In response to a question raised by Parnell *et al.* (2013) “Where to search for hybrids?”, the phenomenon termed “suture zones” was raised. It is worth mentioning the influence of the latest glaciation as presented in the maps of SE Asia by Voris (2000) and Sathiamurthy & Voris (2006) showing the geographical development of the region. It can be said that *Cryptocoryne* habitats have changed drastically during the most recent 20,000 years, and all habitat sites can be considered “suture zones”. Looking at SE Asia today compared to 20,000 years ago, it could be argued that many parts constitute “suture zones”.

The change of the coastline also influenced the inland areas: If one pictures the monumental limestone towers in the Thale Phuket, they would have been situated in a tall evergreen (?) rainforest 20,000–40,000 years ago, with the vegetation looking rather different from what it is today.

The European flora has been rather well known for more than 100 (-200) years. Many botanists have been studying the flora and when they get to know the flora, they will look for hybrids. The genus *Cryptocoryne* has only been studied for 50–60 years and we are now at the knowledge level for “hybrid-detecting”. Within the next 30 years, it is most probable that hybrids from other genera than *Cryptocoryne* will begin to turn up in the Thai flora reports.

Even though much of the above-mentioned discussion of hybrids has concerned species of *Cryptocoryne* outside Thailand, we are convinced that interspecific or infraspecific hybrids occur in Thailand too (Fig. 15, and Idei *et al.*, 2016). But someone has to start looking for them. Eye-catching flowers in general make discovery easier.

The rather large number of *Cryptocoryne* hybrids already encountered implies that hybridization in South-east Asia is a common phenomenon. While vegetative propagation of *Cryptocoryne* provides exceptionally favourable circumstances for establishing and locating hybrid populations like in, e.g., *Nepenthes* and *Potamogeton* (see below), there is no reason to believe that other plant species in SE Asia do not hybridize with frequencies similar to those of *Cryptocoryne*. It is just that *Cryptocoryne* “keeps a record of what has happened” over the previous hundreds of years or more, while many other genera may do likewise and many others do not.

Based on the data from *Cryptocoryne* presented here, the figures mentioned for hybrids within the Flora Malesiana reported by Parnell *et al.* (2013) in which “...almost a third of the estimated 30,000 species have so far been treated but only 12 hybrids have so far been recorded, half of them in *Nepenthes* and *Potamogeton* (three each)...” does not reflect the real situation. The estimate of Parnell *et al.* (2013) “...that there may be at least 1,126 to 1,375 interspecific hybrids in Thailand”, may also be too low.

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