THAI FOREST BULL., BOT. 47(1): 19–28. 2019.
DOI https://doi.org/10.20531/tfb.2019.47.1.05

Natural hybridization – recombination – an ever-ongoing process

NIELS JACOBSEN[1,*] & MARIAN ØRGAARD[1]

ABSTRACT

Exemplified by studies of the SE Asian genus *Cryptocoryne* (Araceae) we provide evidence that: 1) interspecific hybridization is an ever-ongoing process, and introgression and gene exchange takes place whenever physically possible throughout the region; 2) artificial hybridization experiments confirm that wide crosses are possible in a large number of cases; 3) rivers and streams provide numerous, diverse habitats for *Cryptocoryne* diaspores to settle in; 4) the changes in habitats caused by recurrent glaciations resulting in numerous splitting and merging of populations facilitates hybridization and segregation of subsequent generations; 5) hybridization is a major driving element in speciation; 6) populations are the units and stepping stones in evolution – not the species.

KEYWORDS: Araceae, Chromosome numbers, *Cryptocoryne*, hybridization, evolution.

Accepted for publication: 11 January 2019. Published online: 14 February 2019

INTRODUCTION

The completion of *Cryptocoryne* Fisch. ex Wydler for the Araceae volume for Flora of Thailand stands as a milestone after several years of research in this biological unique and complex genus (Boyce *et al.*, 2012). Numerous cultivation experiments, determination of chromosome numbers, morphological studies including SEM studies of the spathe structures and, study of distribution patterns have resulted in many significant taxonomic conclusions (e.g., Arends *et al.*, 1982; Idei *et al.*, 2010, 2017; Bastmeijer, 2018; Jacobsen, 1977, 1985, 1987; Jacobsen *et al.*, 2012, 2015, 2016; Othman *et al.*, 2009, Ørgaard & Jacobsen, 1998; Wit, 1990). Among other findings, long term observations have revealed that interspecific hybridization has occurred more than just a few times (Jacobsen, 1977; Jacobsen *et al.*, 2016; Idei *et al.*, 2017).

An AFLP study of the *Cryptocoryne crispatula* Engl. complex in Thailand and South-East Asian mainland showed that similar morphology does not necessarily reflect genetic similarity (Jacobsen *et al.*, 2015, 2017). This study also revealed a linkage between geographical proximity and genetic similarity. For example, the main course of the Mekong at Chiang Khan, Loei Province, hosts a number of morphologically distinct but genetically closely related hybrids (Idei *et al.*, 2017).

THE GENUS *CRYPTOCORYNE*

The genus presently consists of 64 named species, 18 named varieties, 7 named interspecific hybrids and more than 19 unnamed hybrids.

Cryptocoryne species are aquatic to amphibious perennial herbs with subterranean, creeping and often wide spreading rhizomes. Diaspores are detached and transported freely, ensuring persistence and dispersal of genotypes to numerous niches (Fig. 1). The species inhabit riverbanks and streams, and occasionally forest pools with stagnant water as river levels drop during the dry season (Bastmeijer, 2018; Othman *et al.*, 2009; Jacobsen *et al.*, 2012).

The spathes of *Cryptocoryne* and the sister genus *Lagenandra* Dalzell are unique within the Araceae and ingeniously evolved for fly pollination. A basal kettle encloses the female and male flowers; downwards pointing trichomes of the tube and kettle wall ensure that flies (*Diptera*) cannot climb out of the spathe (tube) having once entered; a flap at the

[1] Section of Organismal Biology, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark.

* Corresponding author: nika@plen.ku.dk

© 2019 Forest Herbarium



Figure 1. *Cryptocoryne albida* Parker on a sandbank and *C. crispatula* Engl. var. *flaccidifolia* N.Jacobsen submerged in the river; Khao Sok River, S Thailand.

kettle entrance effectively traps the flies until the male flowers have matured, then by moving back the flap the flies depart and pick up pollen when passing by the male flowers. Concurrently, the turgor of the trichomes drops and the points collapse into the cell lumen thereby forming a step-like structure for the flies to climb out and continue their task of foraging and pollinating the next spathe. The limb of the spathe emits a strong carrion-like smell to attract flies, and olfactory bodies situated at the top of the female flowers emit a fruity odour, perhaps inviting the flies to feed on the mucilage excreted from cells at the lower part of the kettle wall (Fig. 2).

Main diagnostic features in *Cryptocoryne* are the morphology of the kettle, the tube and limb of the spathe. The limb is highly variable in size, shape and colour, and is characteristic for each species (Fig. 3).

Distribution

Most species of *Cryptocoryne* have a rather restricted distribution (Bastmeijer, 2018; Jacobsen, 1985; Othman *et al.*, 2009; Jacobsen *et al.*, 2012), except for a few species which are proliferous and almost weedy. The mangrove species *C. ciliata* (Roxb.) Fisch. ex Schott is widespread in SE Asia from Bangladesh and eastward to New Guinea, the *C. crispatula* Engl. complex occurs throughout most of mainland SE Asia, and the *C. cordata* Griff. complex through much of the Malesian region (Southern Thailand, Cambodia, Peninsular Malaysia, Sumatra, Natuna Islands and Borneo).

Chromosome numbers

Chromosome numbers vary greatly within species complexes and geographical areas (Arends *et al.*, 1982; Bastmeijer, 2018; Jacobsen, 1977; Wongso *et al.*, 2017). Dysploid series resulting from fusion of chromosomes as well as euploid series are found (Table 1). The chromosome numbers are valuable taxonomic characters as they unequivocally separate and characterize species and groups (Fig. 4).

Mekong taxa

An overview of the *Cryptocoryne crispatula* complex in Thailand was first provided by Jacobsen (1980) and followed up by an AFLP investigation of the complex in mainland SE Asia (Jacobsen *et al.* 2015, 2017). Variation in shape and colours of the spathe limb were found to be linked to genetic markers (Fig. 5).

The Mekong, with a main course of more than 4,300 km, supplemented by countless tributaries and a catchments area of around 795,000 km^2 (about 1.5 times the area of the whole of Thailand), contains an enormous number of habitats. The huge fluctuations in water level challenge the survival ability and adaptability of the *Cryptocoryne* inhabiting the

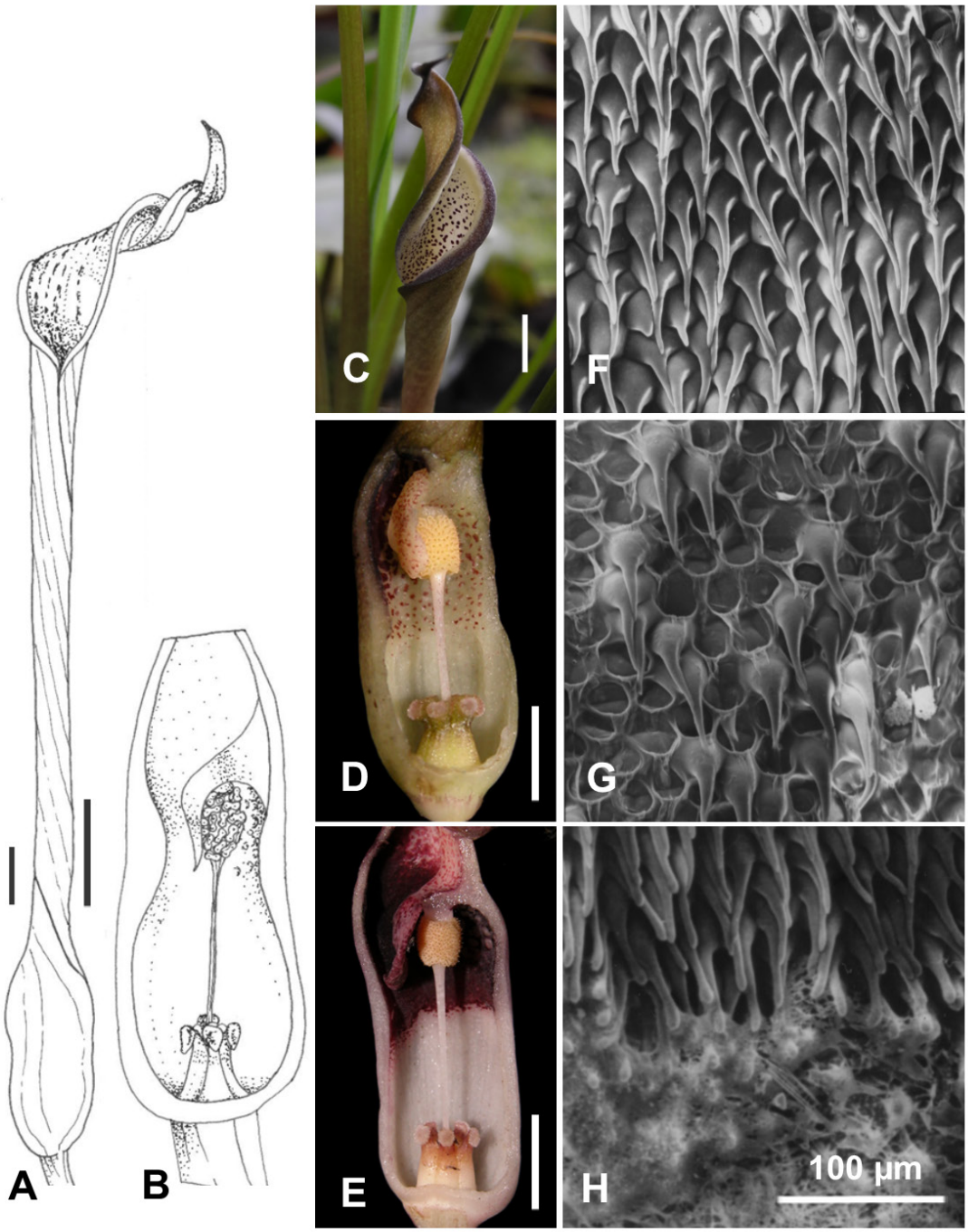


Figure 2. *Cryptocoryne albida* Parker A–C, A. spathe with kettle, tube and limb; B. cut open kettle showing female flowers, sterile spadix part, and flap adjacent to male flowers; C. spathe limb with the characteristic spotted surface; D. *C. crispatula* Engl. var. *kubotae* N.Jacobsen & Bastm., cut open kettle showing female flowers, sterile part of spadix, male flowers, and flap adjacent to male flowers; E. *C. crispatula* Engl. var. *crispatula*, showing the same as D, but the flap has moved upwards to close the exit (Scale bars 0.5 cm); *C. spiralis* (Retz.) Wydl. var. *spiralis*. F–H. F. SEM picture showing downwards pointed trichomes in the tube; G. showing the pointed trichomes sunken into the cell lumen; H. transition zone in kettle with trichomes and mucilage covering (Scale bar 100 µm). Partly after Ørgaard & Jacobsen (1998).

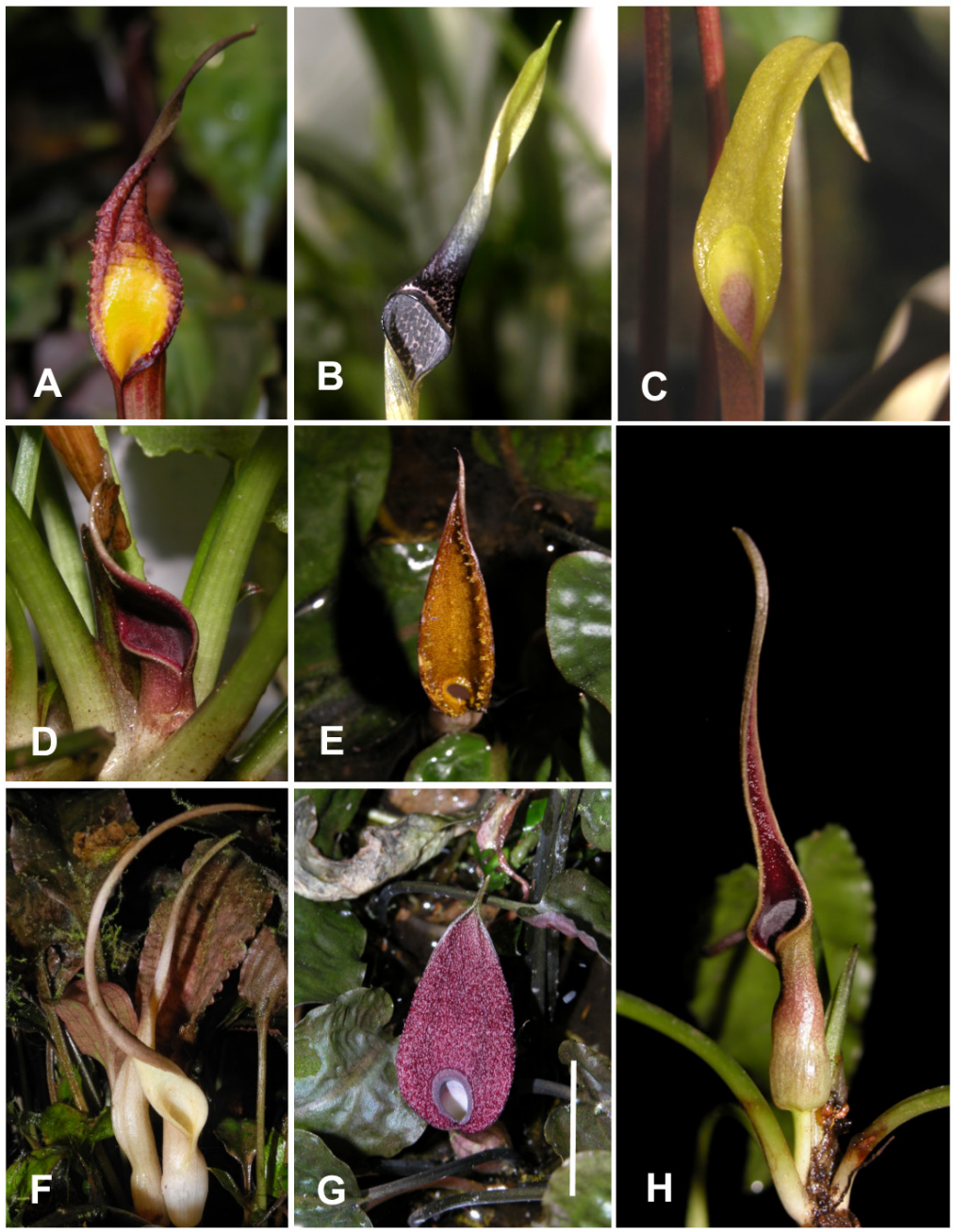


Figure 3. Spathe limbs of different *Cryptocoryne* species. – A. *C. usteriana* Engl., Philippines, 2n = 34; B. *C. nevillii* Hook.f., Sri Lanka, 2n = 28; C. *C. walkeri* Schott, Sri Lanka, 2n = 28; D. *C. matakensis* Bastm. *et al.*, Anambas Islands, 2n = 34; E. *C. bangkaensis* Bastm., South Sumatera Province and the Islands Bangka and Belitung, 2n = 68; F. *C. alba* De Wit, Sri Lanka, 2n = 36; G. *C. griffithii* Schott, southern Peninsular Malaysia, Singapore, Riau Islands and southern Central Kalimantan, 2n = 34; *C. idei* Budianto, Central Kalimantan, 2n = 20. Scale bar 2 cm.

Table 1. Chromosome numbers in *Cryptocoryne* showing the descending dysploid series from 2n = 36 to 2n = 10. Euploid numbers (tetraploids and hexaploids) are found in some groups as well as uneven numbers (triploids and pentaploids). The chromosome numbers in the sister genus *Lagenandra* are 2n = 36, 72.

2n = 36, 54, 72

2n = 34, 51, 68, 85, 102

2n = 30

2n = 28, 42

2n = 26

2n = 22, 33, 44

2n = 33, 66, 88, 110, 132

2n = 20

2n = 14

2n = 10

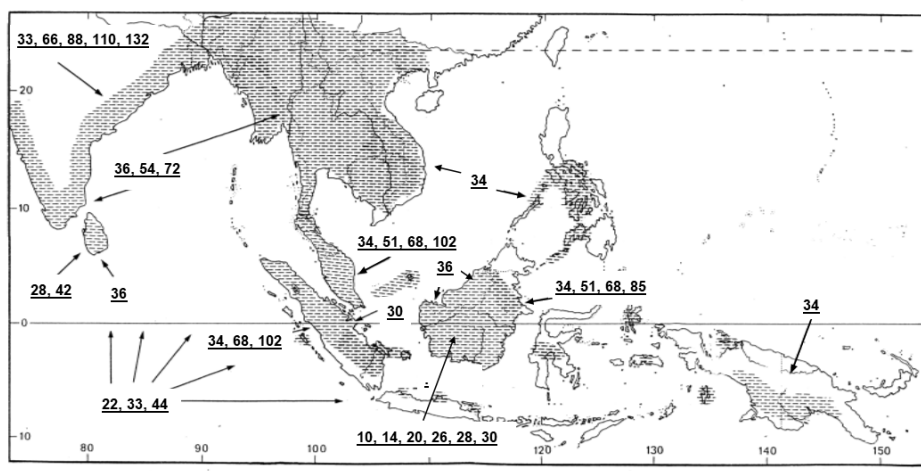


Figure 4. Distribution of the various chromosome numbers in *Cryptocoryne*. Adjusted from Arends *et al.* (1982).

Mekong at Chiang Khan and other places (Idei *et al.,* 2017). Two *Cryptocoryne* taxa from the Mekong at Don Khong, in the Champsak Province of Laos (Idei *et al.,* 2010), and several more hybrids from this region were discussed in detail by Jacobsen *et al.* (2016). *Cryptocoryne* taxa from one of the Mekong tributaries in the Nam Lik Watershed in Laos have been documented (Andersen, *et al.* 2006).

Additional examples of naturally occurring hybrids from Sri Lanka, Peninsular Malaysia, Sarawak, and Kalimantan were also detailed by Jacobsen *et al.* (2016).

Artificial hybrids

The *Cryptocoryne* studies have included a large number of artificial interspecific crossings, resulting in more than 80 F_1 generations of which a few are presented in Jacobsen *et al.* (2016). The hybridization experiments show that it is possible to obtain an overall fruit set of more than 30%, yielding viable seedlings of almost 10%, which is comparable to other similar experiments in *Hordeum* L. (Bothmer & Jacobsen, 1986, 1991), *Brassica* L. (Bothmer *et al.*, 1995), and *Crocus* L. (Jacobsen *et al.*, unpubl.).

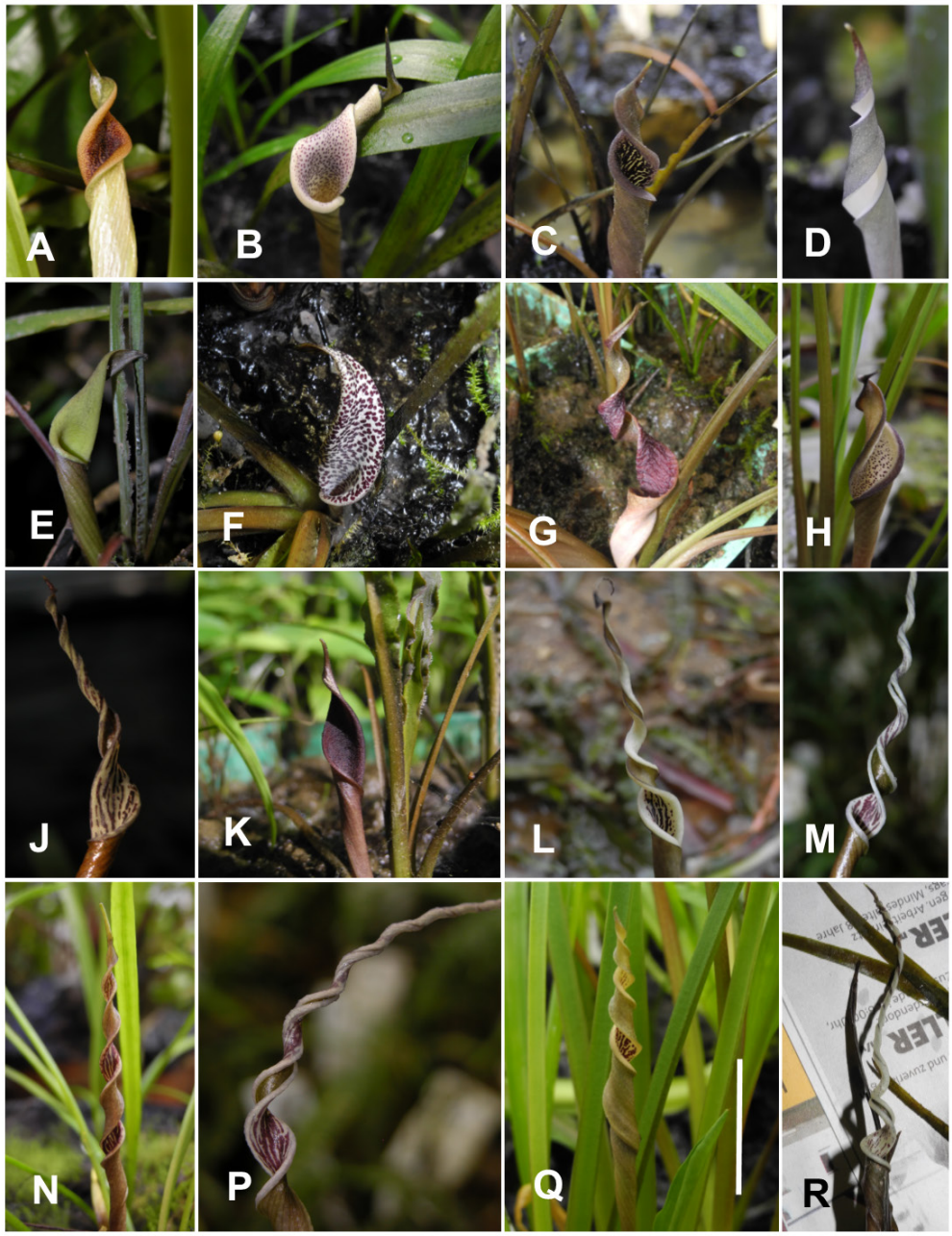


Figure 5. Spathe limbs of the *Cryptocoryne crispatula* Engl. complex. – A. *C. crispatula* var. *yunnanensis* (H.Li) H.Li & N.Jacobsen, Ban Phon Gun Nam Ken, central Laos; B. *C. crispatula* var. *crispatula* (*albida* like), Nam Cheng, central Laos; C. *C. crispatula* var. *crispatula* (short spathe tube), Kaeng Kood Koo, NE Thailand; D. *C. crispatula* var. *planifolia* H.Zhou *et al.*, southeastern China; E. *C. crispatula* hybrid, Chiang Khan, NE Thailand; F. *C. crispatula* var. *decus-mekongensis* T.Idei *et al.*, Don Khon, southern Laos; G. *C. mekongensis* T.Idei *et al.*, Don Khon, southern Laos; H. *C. albida* Parker, Klong Naka G. S., PEN Thailand; J. *C. crispatula* var. *crispatula* (long spathe tube), Don Khon, southern Laos; K. *C. loeiensis* Bastm. *et al.*, Chiang Khan, NE Thailand; L. *C. crispatula* var. *balansae* (Gagnep.) N.Jacobsen, Huu Lien, northeastern Vietnam; M. *C. crispatula* var. *flaccidifolia* N.Jacobsen, Khao Sok River, PEN Thailand; N. *C. crispatula* var. *kubotae* N.Jacobsen & Bastm., Pha Taem, E Thailand; P. *C. crispatula* var. *balansae*, Khao Sok River, PEN Thailand; Q. *C. albida* (*crispatula* like), 3 Pagoda Pass, SW Thailand; R. *C. crispatula* var. *tonkinensis* (Gagnep.) N.Jacobsen, Hai Ha, northeastern Vietnam. Scale 2 cm.

Besides genetic malfunctioning in some hybrid combinations, methodological errors and other factors no doubt reduced the number of hybrids obtained. These include: 1) pollen not applied at the receptive stage of the female flowers; 2) pollen not mature; 3) pollen not applied correctly to the stigmas; 4) fruit overlooked resulting in seeds rotting or eaten; 5) seeds and seedlings not properly cultivated; 6) fruit and seed development ceased due to external factors.

In nature, more than 25% of the currently recognized *Cryptocoryne* taxa are of hybrid origin (Jacobsen *et al.*, 2016). Although some of the hybrids have reduced fertility and seed set, the hybrid genotype is persistent and vegetatively proliferated, forming large populations spread by stolons.

THE NATURAL HABITATS

Running water (with a few exceptions) is a prerequisite for the occurrence of *Cryptocoryne* in nature. On a map, the Mekong does not show the smaller tributaries, including *Cryptocoryne* habitats and all the habitats that actually exist or have existed before modern agriculture. The Department of Irrigation & Drainage Sarawak (2017) has produced a series of maps of the river systems/catchments in the northern Borneo state of Sarawak (Fig. 6), also including detailed maps of the specific catchment areas, e.g. the Lupar Basin. Figure 6 reveals an astonishing number of small streams including habitats suitable for *Cryptocoryne*. Even many of the white areas on the map are actually interwoven with streamlets and ditches, not detectable at the map scale. The white areas are to a large extent lowland forest peat swamp areas, where the water percolates through the forest floor in countless small canals and streamlets, partly caused by the tidal movements of the fresh water zone.

In the Lupar Basin, eight species of *Cryptocoryne* have been reported: *C. ciliata* var. *ciliata*, *C. cordata* var. *grabowskii* (Engl.) N.Jacobsen, *C. fusca* De Wit, *C. lingua* Engl., *C. longicauda* Engl., *C pallidinervia* Engl., *C. striolata* Engl. and *C. uenoi* Yuji Sasaki. Not all catchment areas in Sarawak have as many species as the Lupar, however, it must be taken into consideration that only small areas of Sarawak have yet been searched for *Cryptocoryne*.

THE *CRYPTOCORYNE* HABITATS IN PREVIOUS TIMES

The present day land areas in SE Asia are considered stable. However, about 70,000–20,000 years BC, the ocean water level was more than 100 m lower than today (Fig. 7). The ocean floor between Vietnam, Cambodia, Thailand, Peninsular Malaysia,

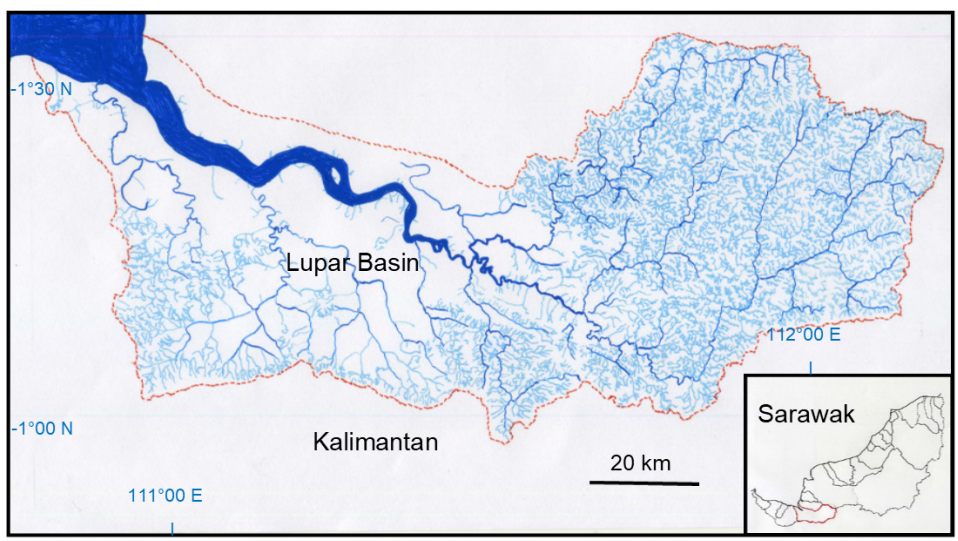


Figure 6. The Lupar Basin, showing its location in Sarawak. Redrawn after Department of Irrigation & Drainage Sarawak (2017).

Sumatra and Borneo was part of a large land area joining the above-mentioned countries into the so-called Sunda Shelf (Sathiamurthy & Voris, 2006; Voris, 2000). Figure 7 shows Asia around 20,000 years BC, depicting the larger rivers in the region. Melting of the last glaciation ice caused a rise of the sea level by more than 100 m resulting in radical changes of habitats for many species, including *Cryptocoryne*. Plant populations were either wiped out or had to adjust to the receding river levels.

Around eight glaciation and inter glaciation periods have occurred during the last 800,000 years (EPICA, 2004), generating dramatic climate changes. These environmental fluctuations have required species to adapt, thereby climate being a major evolutionary driving factor. Recurrent water level fluctuations, resulting in extensions and contractions of river systems, have forced *Cryptocoryne* populations (and all other plants) up-river and down-river to colonize new streams thereby meeting other populations, i.e. new possible hybridization partners. Climate changes act as a major driving force in hybridization and recombination – a driving force in plant speciation.

CONCLUSION

River systems are dynamic elements, and influenced by humans, e.g. land cultivation.

Today *Cryptocoryne longicauda* occurs in Johor (Peninsular Malaysia), Sumatra, the Riau Islands of Bintan, Singkep, Bangka, and Borneo (Sarawak and west Kalimantan). The evolutionary unit is not the "species" *C. longicauda*, but the individual populations, all of them being subjected to different selection pressures. The *C. crispatula* complex (e.g. Jacobsen *et al.*, 2012, 2016; Idei *et al.*, 2017) is an excellent example of how thousands of different niches found within the distribution area provide the basis for the proliferation of the different genotypes brought about by hybridization. The evolutionary units are populations (Erlich & Raven, 1969).

In relation to the changing climates over the last 600,000 years, it is remarkable that there is a strict correlation between the CO_2 content in the atmosphere and the temperature (EPICA, 2004). The temperature fluctuates around 5°C, and the CO_2 concentration varies between 200 ppm during glaciations and 300

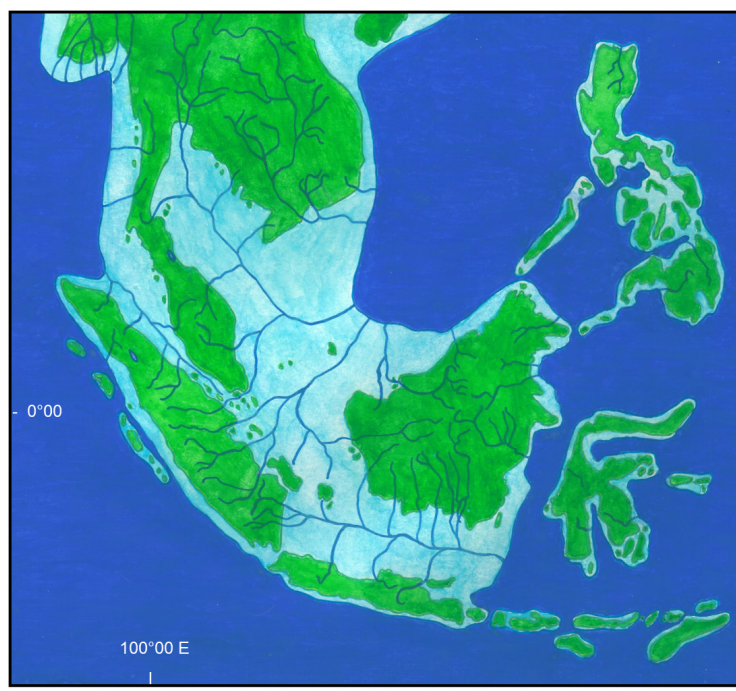


Figure 7. Map of SE Asia (present day in green) showing the exposed Sunda Shelf during the last glaciation (light blue and green) with the present river systems and their extensions. Redrawn after Sathiamurthy & Voris (2008).

ppm during warmer periods. An alarming development is that in 2017 the CO_2 concentration has reached 400 ppm (ESRL, 2017), indicating a forthcoming dramatic rise in temperature, a new upcoming change that will influence the selection pressures on *Cryptocoryne*, as well as on all living things.

ACKNOWLEDGEMENTS

Karen Rysbjerg Jensen skillfully assisted in preparation of figure 7. Many friends have over the years shared their live collections, making the many colour photographs possible. Jacob Weiner kindly reviewed the English text.

REFERENCES

Andersen, T., Pedersen, O., Christensen, C. & Jacobsen, N. (2006). *Cryptocoryne* of the Nam Lik Watershed in Northwestern Laos. The Aquatic Gardener 19(1): 24–32.

Arends, J.C., Bastmeijer, J.D. & Jacobsen, N. (1982). Chromosome numbers and taxonomy in *Cryptocoryne* (Araceae) II. Nordic Journal of Botany 2(5): 453–463.

Bastmeijer, J.D. (2018). The crypts pages. – Published at http://crypts.home.xs4all.nl/*Cryptocoryne*/index.html [Accessed November 27th, 2017].

Bothmer, R. von, Gustafsson, M. & Snogerup, S. (1995). *Brassica* sect. *Brassica* (Brassicaceae) II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. Genetic Resources and Crop Evolution 42: 165–178.

Bothmer, R. von & Jacobsen, N. (1986). Interspecific crosses in *Hordeum* (Poaceae). Plant Systematics and Evolution 153: 49–64.

_____. (1991). Interspecific Hybrids within the Genus Hordeum. p. 411–431. In P.K. Gupta and T. Tsuchiya ed.: Chromosome Engineering in Plants: Genetics, Breeding, Evolution. Elsevier. Part A.

Boyce, P.C., Sookchaloem, D., Hettterscheid, W.D.A., Gusman, G., Jacobsen, N., Idei, T., & Du, NV. (2012). Flora of Thailand, Acoraceae & Araceae, Vol. 11, 2, p. 218–232 & Plates XLV–LVIII.

Department of Irrigation & Drainage Sarawak. (2017). http://www.did.sarawak.gov.my/modules/web/pages.php?mod=webpage&sub=page&id=320 [Accessed November 27th, 2017].

EPICA. (2004). Eight glacial cycles from an Antarctic ice core. Nature 429: 623–628. https://www.nature.com/articles/nature02599 [Accessed November 27th, 2017].

Erlich, P.R., & Raven, P.H. (1969). Differentiation of Populations. Science 165: 1228–1232.

ESRL. (2017). Trends in Atmospheric Carbon Dixoide. https://www.esrl.noaa.gov/gmd/ccgg/trends/ [Accessed November 27th, 2017].

Idei, T., Bastmeijer, J.D. & Jacobsen, N. (2010). Geschichten vom Mekong: Zwei neuen Cryptocorynen (Araceae). Aqua Planta 35(4): 139–146.

_____. (2017). Stories from the Mekong, part 2: The *Cryptocoryne* (Araceae) habitats in the Chiang Khan district, Loei province, Thailand. Thai Forest Bulletin (Botany) 45(1): 58–78.

Jacobsen, N. (1977). Chromosome numbers and taxonomy in *Cryptocoryne* (Araceae). Botaniska Notiser 130: 71–87.

_____. (1980). The *Cryptocoryne albida* group of Mainland Asia. Mededelingen Landbouwhogeschool Wageningen 19: 183–204.

_____. (1985). The *Cryptocoryne* of Borneo (Araceae). Nordic Journal of Botany 5: 31–50.

_____. (1987). *Cryptocoryne*. In M.D. Dassanyake & F.R. Forsberg (eds). A revised Handbook to the Flora of Ceylon. IV: 85–99.

Jacobsen, N., Idei, T. & Sookchaloem, D. (2012). '11. *Cryptocoryne*', in: P.C. Boyce, D. Sookchaloem, W.D.A. Hettterscheid, G. Gusman, N. Jacobsen, T. Idei, & N.V. Du. Flora of Thailand, Acoraceae & Araceae, Vol. 11, 2, p. 218–232 & Plates XLV–LVIII.

Jacobsen, N., Bastmeijer, J.D., Christensen, C., Idei, T., Lange, C.A., Orabi, J., Sookchaloem, D., Toneato, F. &. Ørgaard, M. (2015). The use of AFLP markers to elucidate relationships within *Cryptocoryne* (Araceae). Aroideana Vol. 38E (1): 186–193.

Jacobsen, N., Bastmeijer, J.D., Bogner, J., Budianto, H., Ganapathy, H.B., Idei, T., Ipor, I.B., Komala, T., Othman, A.S., Rosazlina, R., Siow, J., Wongso, S., & Ørgaard, M. (2016). Hybrids and the Flora of Thailand revisited: Hybridization in the Southeast Asian genus *Cryptocoryne* (Araceae). Thai Forest Bulletin (Botany) 44(1): 53–73.

Jacobsen, N., Bastmeijer, J.D., Christensen, C., Idei, T., Lange, C.A., Orabi, J., Sookchaloem, D., Toneato, F. & Ørgaard, M. (2017). *Cryptocoryne* crispatula (Araceae) in Thailand seen with the help of AFLP glasses. 17th Flora of Thailand Conference, 21–25 August 2017, Krabi. E-Poster: 59, p. 143.

Ørgaard, M. & Jacobsen, N. (1998). SEM study of the surface structures of the spathe in *Cryptocoryne* and *Lagenandra* (Araceae: Aroideae: *Cryptocoryne*ae). Botanical Journal of the Linnaean Society 126: 261–289.

Othman, A.S., Jacobsen, N. & Mansor, M. (2009). *Cryptocoryne* of Peninsular Malaysia. Penerbit University Sains Malaysia, Pulau Pinang.

Sathiamurthy, E. & Voris, H.K. (2006). Maps of Holocene sea level transgression and submerged lakes on the Sunda Shelf. - Natural History Journal, Chulalongkorn Univ. Suppl. 2: 1–43.

Voris, H.K. (2000). Maps of Pleistocene sea levels in South-east Asia: shorelines, river systems and time durations. Journal of Biogeography 27: 1153–1167.

Wit, H.C.D. de. (1990). Aquarienpflanzen, 2. Auflage. Ulmer, Stuttgart.

Wongso, S., Bastmeijer, J.D., Budianto, H., Ipor, I.B., Jensen, K.R., Ørgaard, M. & Jacobsen, N. (2017). Six new *Cryptocoryne* taxa (Araceae) from Kalimantan, Borneo. Willdenowia 47(3): 325–339.