# The use of AFLP markers to elucidate relationships within *Cryptocoryne* (Araceae)

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## ABSTRACT

Four species complexes in *Cryptocoryne* were included in a study of genetic diversity: The *C. beckettii* complex (Sri Lanka), the *C. crispatula* complex (Mainland Asia), and selected accessions from the di- and polyploid cordate leaved species from Malesia, altogether comprising 400+ accessions. The Amplified Fragment Length Polymorphism (AFLP) method was discriminative not only within but also between species complexes. Within the cordate leaved Malesian species, it was also possible to discriminate between diploids, tetraploids and hexaploids forming very robust relationship groupings. The AFLP study also revealed that, geographical proximity exposed closer genetical relationship than morphologically similar accessions geographically apart.

## INTRODUCTION

The about 55 species of *Cryptocoryne* are aquatic to amphibious herbs with far creeping and proliferously dividing subterranean rhizomes, thereby enabling them to form large stands in streams and rivers. The shape of the leaves and the shape and colours of the limb of the spathe are important diagnostic taxonomic characters (Bastmeijer, 2015).

*Cryptocoryne* that live in larger river systems are exposed to innumerable habitats/niches (see e.g. **Figure 2)** and these are principally connected with each other.

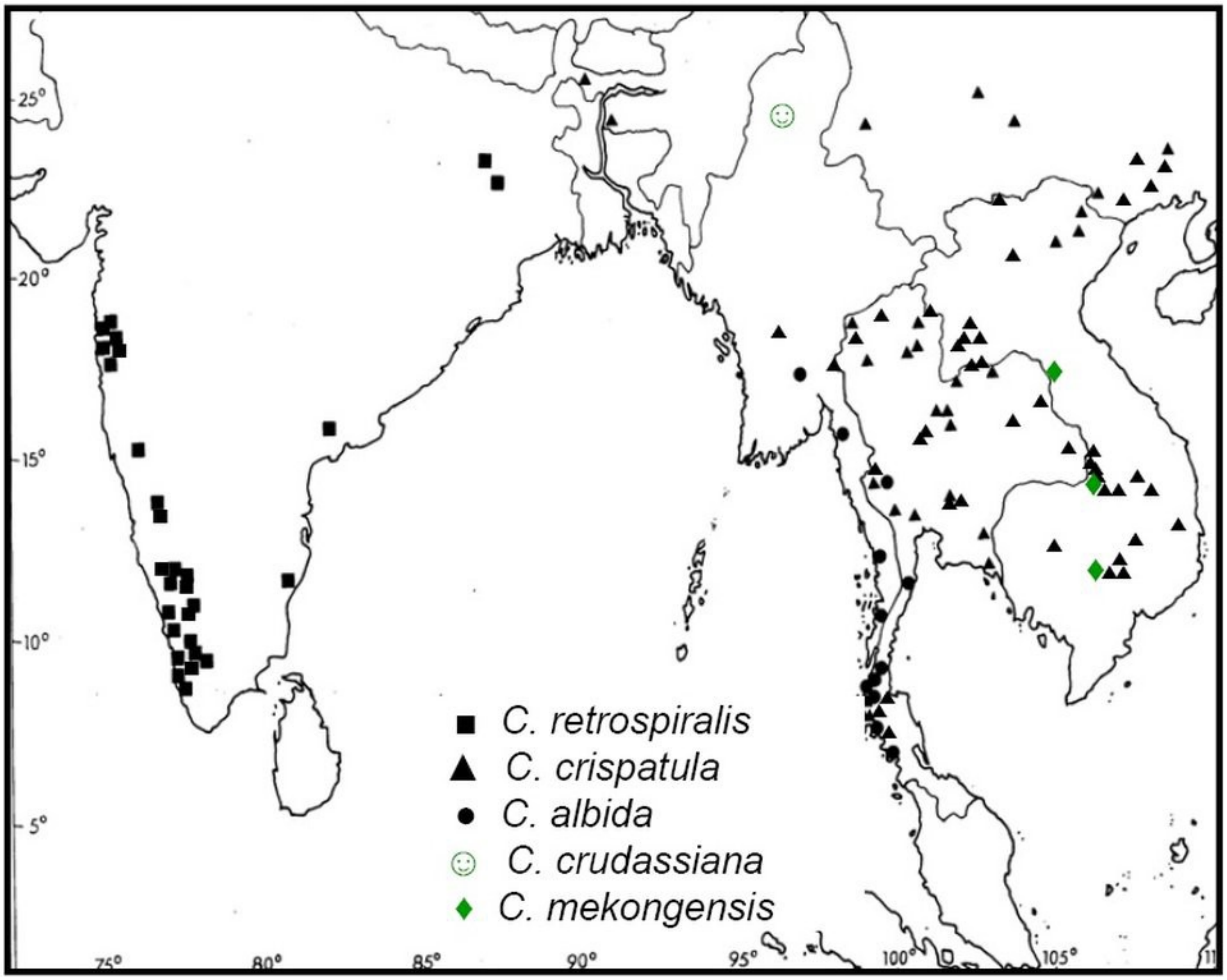


**Figure 1.** Distribution of the *Cryptocoryne crispatula* complex in south-east Asia.

Seeds and rhizome fragments may be transported freely within river systems, and where species are mixed in populations they may hybridize and exchange genes stochastically within operation distance of the pollinating flies.

*Cryptocoryne* genotypes can theoretically "exist for ever" by means of vegetative reproduction; over time viable hybrids will therefore accumulate.

More than 25% of the about 91 named and unnamed *Cryptocoryne* have proven to be of hybrid origin (54 species, an additional 12 varieties and 25 natural hybrids).

## MATERIALS AND METHODS

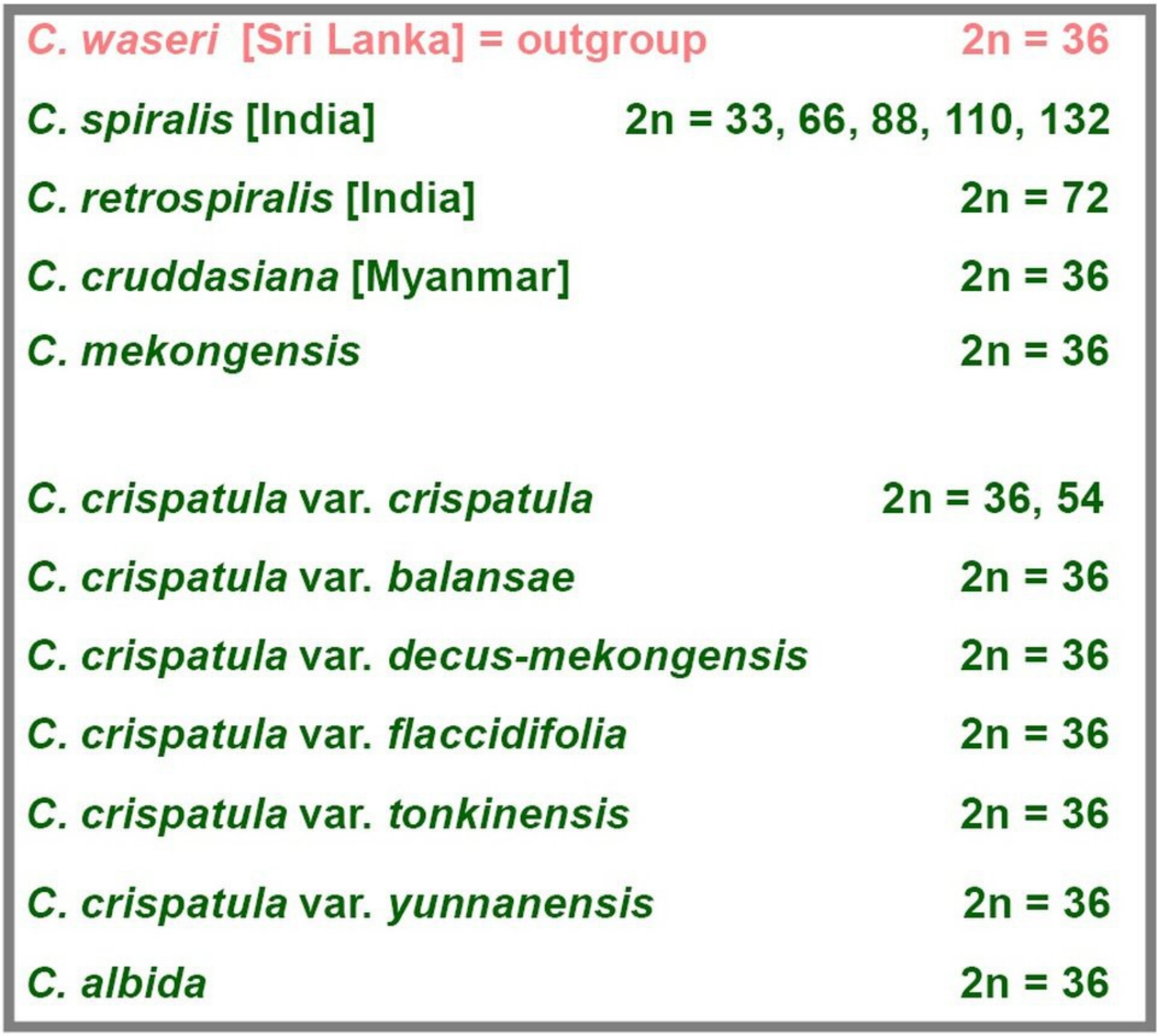
Population structure (**Figure 5)** was analyzed with a Bayesian based clustering approach using 'STRUCTURE' v.2.3 software (Pritchard et al., 2009). In order to find the best group (K) number, the number of groups (K) was tested from K=2 to

**Figure 2.** *Cryptocoryne* habitat in southern Thailand showing a stand of *C. albida* Parker on top of the sandbank and *C. crispatula* Engler *var. flaccidifolia* N. Jacobsen in the water around the sandbank. Khao Sok N.P.

K=12 with 20 replicates of each run and a 'burn-in' period of 10,000 iterations and 10,000 Markov chain Monte Carlo (MCMC) iterations. The optimal number of groups was determined according to Evanno et al. (2005). Finally, ten replicates with the optimal number of groups were run at a 'burn-in' period and MCMC iterations of 100,000. Based on Jaccard's genetic distance, principal component analysis (PCO) was used to visualize the genetic distance between the four groups inferred by the STRUCTURE analysis. For this purpose, the R language and environment software (v.2.11.1; R Development Core Team, 2011) including the 'Modern Applied Statistics with S-plus' package was employed (Venables and Ripley, 2002).

## RESULTS

In the ongoing molecular studies of variation and evolution of the genus *Cryptocoryne,* it was decided to study four species complexes in more detail: The *C. beckettii* Trim. complex (Sri Lanka), the *C.*



| | |
|---|---|
| *C. waseri* [Sri Lanka] = outgroup | 2n = 36 |
| *C. spiralis* [India] | 2n = 33, 66, 88, 110, 132 |
| *C. retrospiralis* [India] | 2n = 72 |
| *C. cruddasiana* [Myanmar] | 2n = 36 |
| *C. mekongensis* | 2n = 36 |
| *C. crispatula* var. *crispatula* | 2n = 36, 54 |
| *C. crispatula* var. *balansae* | 2n = 36 |
| *C. crispatula* var. *decus-mekongensis* | 2n = 36 |
| *C. crispatula* var. *flaccidifolia* | 2n = 36 |
| *C. crispatula* var. *tonkinensis* | 2n = 36 |
| *C. crispatula* var. *yunnanensis* | 2n = 36 |
| *C. albida* | 2n = 36 |

**Figure 3.** *Cryptocoryne* taxa of the *C. crispatula* complex included in the Fig. 6 AFLP investigation.

*crispatula* Engl. complex (Mainland Asia; **Figure 1;** Jacobsen & al., 2012), and selected accessions from the di- and polyploid cordate leaved species from Malesia (*C. cordata* Griff. etc.), altogether comprising 400+ accessions.

The Amplified Fragment Length Polymorphism (AFLP) study proved capable of not only being able to discriminate within the species complexes, but it was also able to cope with all 400+ accessions in one run, discriminating the *C. beckettii* group, the *C. crispatula* group, and, within the cordate leaved Malesian species, it was able to discriminate between diploids, tetraploids and hexaploids forming very robust relationship groupings.

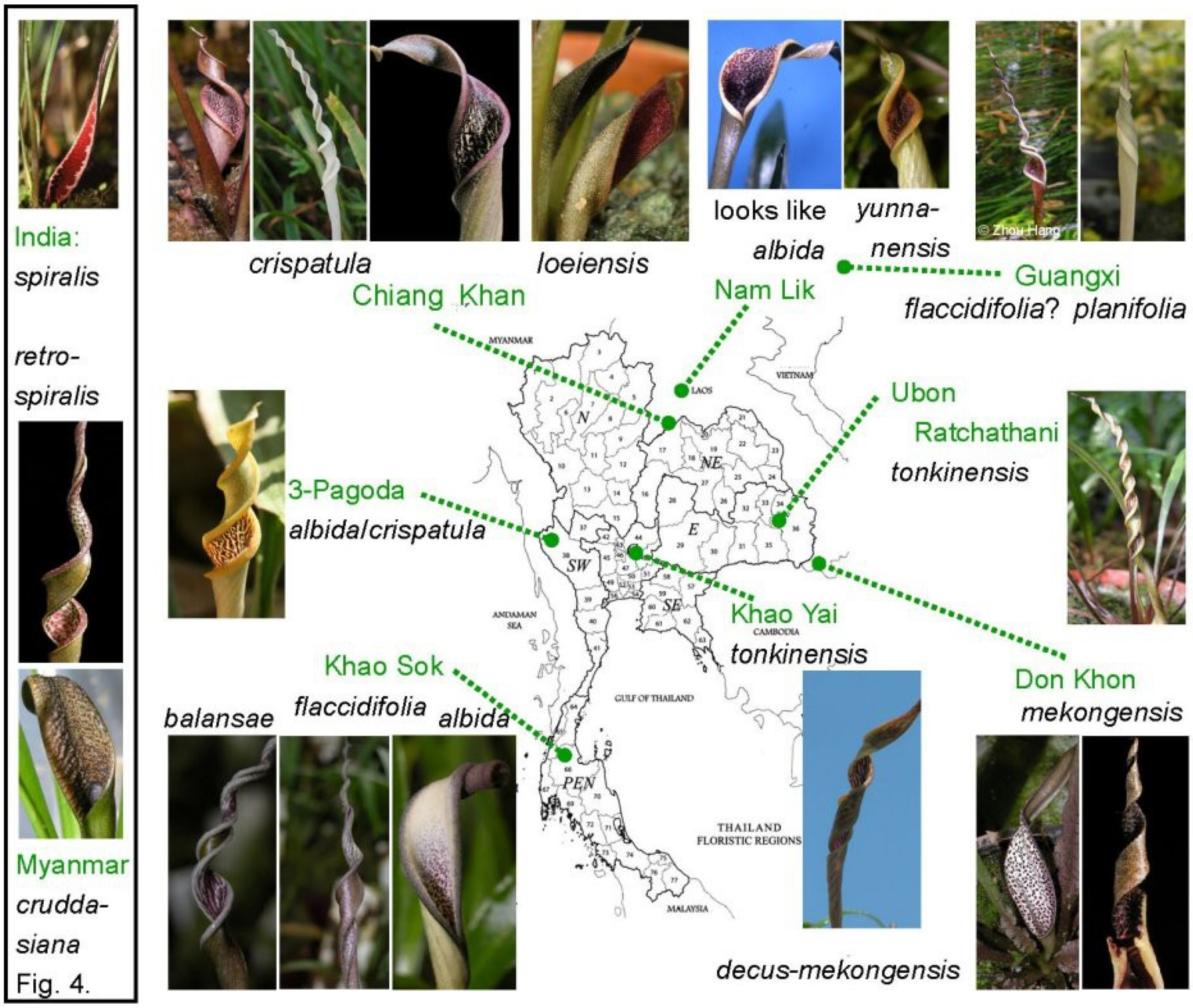


**Figure 4.** Examples of the species in the *Cryptocoryne crispatula* group centered around Thailand.

The AFLP study also showed that, geographical proximity revealed closer genetical relationship contrasting morphologically similar accessions geographically further apart.

The PCO analysis separated the collection into four major complexes: 1) Sri Lanka, 2) Malesia (diploids), 3) Malesia (tetra- and hexaploids) and 4) Mainland Asia (**Figure 5)**.

The graph in **Figure 6** depicts the genetic distances between the accessions of group 4, the *C. crispatula* complex (**Figure 3)**, as shown in **Figure 4**. The names are based on the morphology of the leaves and the limb of the spathe. The diagram shows that there are shorter genetic distances within geographical regions than there are ”within” names, i.e. *flaccidifolia* and *balansae* from from Khao Sok are closer to each other than e.g.

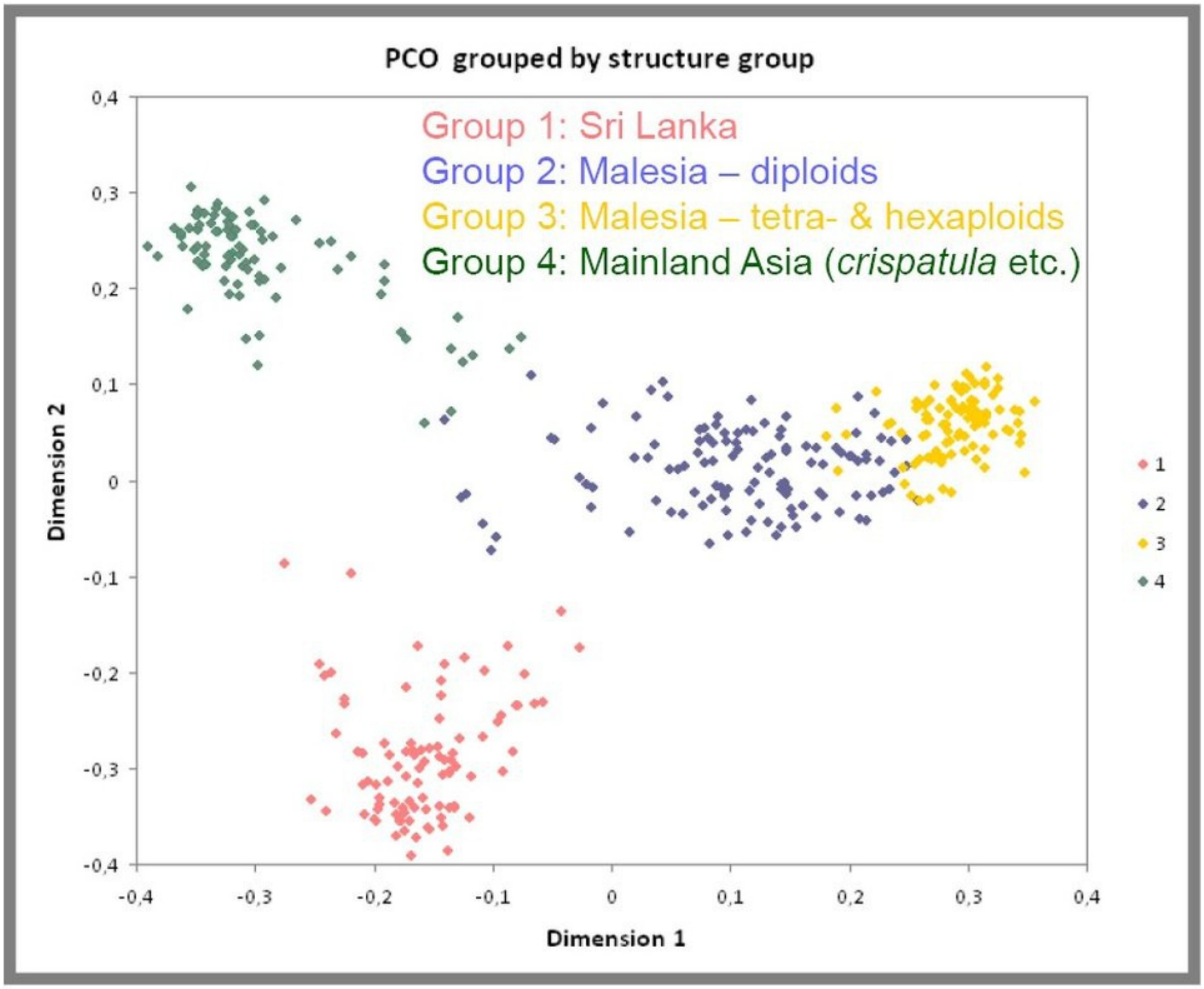


**Figure 5.** Population structure was analyzed using 'STRUCTURE' v.2.3 software resulting in four groups. Principal component analysis (PCO) was used to visualize Jaccard's genetic distance between the four groups. The PCO analysis separated the collection into four major complexes: 1) Sri Lanka, 2) Malesia (diploids), 3) Malesia (tetra- and hexaploids) and 4) Mainland Asia.

the *balansae* from Khao Sok and Khao Yai are.

## DISCUSSION

We often think and talk about "species" as if they exist as natural evolutionary units. The word "species" means "an outward appearance" – a "look" – a kind.

And as "kinds" they exist and the populations of different kinds – whatever kind they may be – genotypes – will sexually reproduce – and when the different kinds look different – we say that they hybridize.

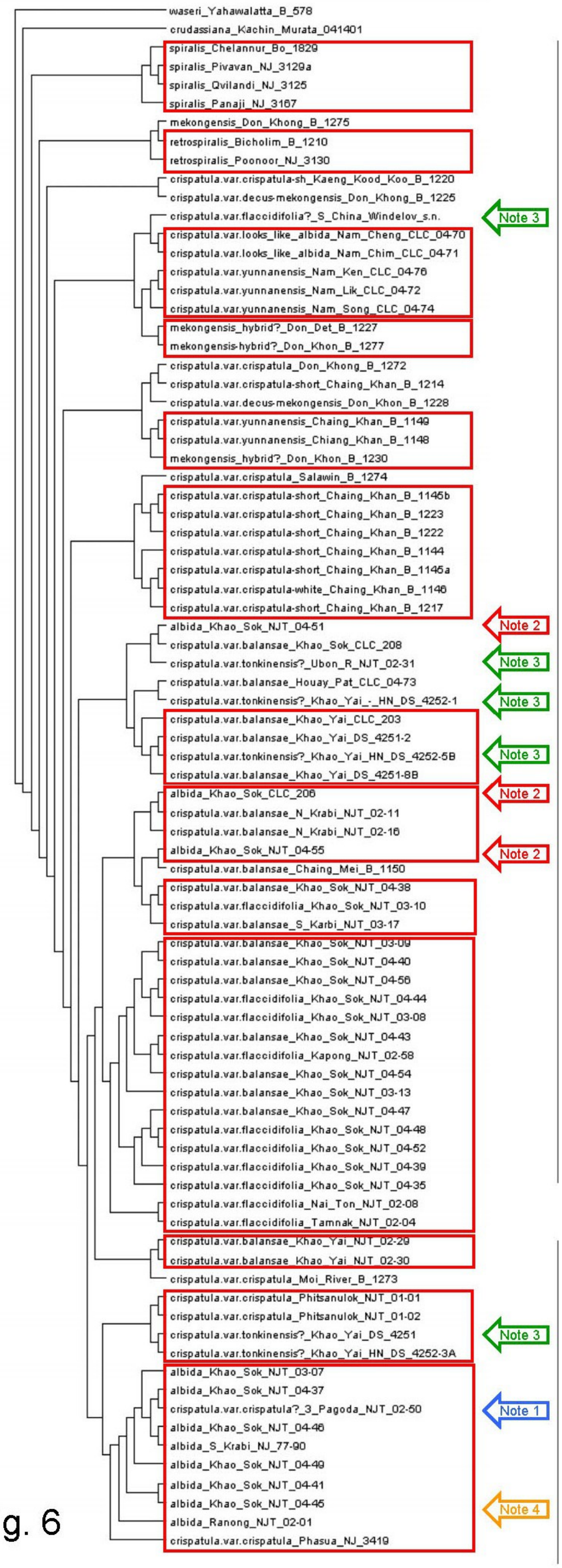


Fig. 6

**Figure 6.** Genetic distances between accessions of group 4, the *C. Crispatula* complex, as shown in **Figure 5.** Interestingly there are shorter genetic distances between taxa sharing habitats, i.e. *flaccidifolia* and *balansae* from Khao Sok than there are ”within taxa”, e.g. The *balansae* accessions from Khao Sok and Khao Yai.

**Note 1.** The *crispatula* in the *albida* grouping most likely represents an *albida* with *crispatula* like traits (i.e. spathe limb form).

**Note 2.** The three *albida* found away from the other *albida* accessions in association with *balansae* (Khao Sok, N Krabi & Chiang Mai) may represent plants of hybrid origin (not recognized on the basis of their morphology).

**Note 3.** The geographically different accessions named *tonkinensis* seem to be “unrelated”, and the very narrow leaved “*flaccidifolia*” from China could perhaps just as well have been named *tonkinensis* (based on the 2–3 mm wide leaves).

**Note 4.** The diagram also clearly shows that what has been called *C. albida* clearly falls within *C. crispatula* in a broad sense.

Species are traditionally considered as the natural evolutionary units. However, this study strongly supports the view that it is in the populations evolution takes place and that they should be regarded as the naturally evolutionary centres.

*Cryptocoryne* can be considered as numerous genetically different populations living in different river systems. Hybridization is a driving evolutionary force which continuously produce new genotypes which subsequently are dispersed randomly in the river systems.

“Species” as such cannot be considered as the natural evolutionary unit, but what we see are populations that are the results of random/stochastic events brought about by the “historical” events etc. etc. - - “Species” are the “leftovers”.

## ACKNOWLEDGEMENTS

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