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Morphometric and morphological analyses of leaves in two species of Araceae : *Montrichardia linifera* and *Montrichardia arborescens* among different French Guiana populations

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Abstract : In French Guiana, Araceae represents one of the most important monocots family. *Montrichardia* is one of its 117 genera and it is represented by two species, *M. arborescens* and *M. linifera*. Currently, the two species can be found meanly in diverse sites of the region. Recent studies on the species have failed to establish clear geographical patterns between the two species and among their populations. Here, the morphological variations of leaves were studied in the two species of *Montrichardia* using classical measurements of leaf outlines shape. In order to identify intra-populational variabilities and interspecific differences of their leaves, the Elliptic Fourier Analysis and multivariate statistical analyses were performed. The results obtained showed that the morphological variables were more discriminating at interspecific level. At the intra-populational level, there are two parameters to consider. Firstly, in *M. arborescens*, morphological variables had a more important part in the population discrimination than morphometric variables. Secondly, in *M. linifera*, the populations were better discriminated by morphometrics variables than for *M. arborescens*. Moreover, it was highlighted that even if differences could be observed between both species within each site, one population of one species from one site could be morphometrically and/or morphologically similar to another population of the other species in another site. This result suggests that the variation of the leaf studied traits was as important within site between the two species as among sites within a given species.

Key Words. Araceae, French Guiana, Leaf measures, *Montrichardia*, Elliptic Fourier Analysis.

Introduction

The rain forest term is reserved to the wettest type forest which is characterized by its sempervirence, a greater abundance in epiphytes, and a particularly short or absent dry season (Schnell, 1987).

Lowland tropical rain forests are the most biologically diverse biome in the world and it has a very high species diversity (Clark *et al.*, 1999). The richest forests in Amazonia and Southeast Asia, were found to exhibit 1000 trees species per square kilometer (Thomas & Baltzer, 2002). The French Guiana is located within this tropical forest type which covers a third of the South American continent and part of the Northeastern province (Atlantic) (Richard-Hansen & Le Guen, 2001). French Guiana is covered by more than 95% of lowland tropical rain forests and these forests heritage is almost intact (Sabatier & Prévost, 1990).

The Araceae family has 117 genera and 3794 species (Boyce and Croat, 2011). This is one of the most important families of monocots, the third most diverse in French Guiana (Herrera, 2008). This family occurs in every continent excepted Antartica, but it is mainly tropical (Grayum, 1990). This is the predominant family of herbaceous hemiepiphytic climbers with several terrestrial or swamp herbs and a few genera of floating aquatic plant (*Pistia* and “Lemnaceae”) (Gentry, 1993; Nauheimer *et al.*, 2012). Within the Araceae, the genus *Montrichardia* was described for the first time by H. Crüger in 1854. It includes helophyte plants which can be found in wetlands, in Latin America, from Mexico to Rio de Janeiro, in Brazil (Mayo *et al.*, 1997; Croat *et al.*,2005), *Montrichardia* is entomophilous and beetle pollinated

(GIBERNAU *et al.*, 2003). Currently two species can be recognized in this genus: *Montrichardia arborescens* (L.) Schott and *Montrichardia linifera* (Arruda) Schott (CROAT *et al*.,2005). Recently, an extinct species was found in Colombia, *Montrichardia aquatica* (HERRERA, 2008).

In terms of ecology, *M. arborescens* is an evergreen herb which grows in fresh-water habitats, in swamps, and along the rivers by forming dense populations (GIBERNAU, 2003).

Several studies have been made on *Montrichardia.* SILVA *et al.* (2012) compared the variation of leaf characters among populations of *M. linifera* with geometrical morphometrics by using landmarks of the leaf blade. Other methods of leaf morphometric comparison have been used on other Aroid taxa, such as the Elliptic Fourier Analysis (EFA) of leaf outline shape (ANDRADE *et al.*, 2008; 2010). This method has never been used for *Montrichardia* and, in their study, ANDRADE *et al.* (2010) specified that EFA could be a useful tool to distinguish Araceae species.

Here, the morphological variations of leaves were studied of leaves in the two species of *Montrichardia* using classical measurements of leaf outlines shape. Leaf morphologies were compared at two levels, first interspecific (differences between the two species), and second intraspecific (differences among the populations) on both species. The approach was to sample different populations from the northwest (Saint Laurent du Maroni) to the northeast (Saint Georges) of French Guiana.

Materials and methods

Plant materials

This study takes place in seven different sites in French Guiana where seven populations of *Montrichardia arborescens* and five populations of *Montrichardia linifera* were sampled. For each site GPS coordinates were taken (Fig. 1, Table 1). Populations sampled were either close to a city on the side of a road (Cayenne, Kourou, Sinnamary, St Georges, St Laurent du Maroni) or in a place with less urban activities (Maripa, Kaw's swamp). Twenty individuals were sampled when possible in each population (see Table 1). In total, two hundred and ten individuals were sampled with 120 *M. arborescens* and 90 *M. linifera*. For each individual, one mature leaf, in general the second or third below the apex, was collected. Damaged or incomplete blades were avoided. Plants were chosen with a relative distance between them (1 – 2 m) to reduce the risk of sampling the same clone. Each collected leaf was pinned onto a horizontal white board alongside a 30 cm measuring rule scale and a picture was taken with a digital camera (Olympus E-PL2). One of the possible biases was the undulations to various degrees of the leaf margin. To reduce this bias, leaf margin was pinned as frequently as necessary and photographies were taken directly overhead the leaf.

Once photographed, morphological measures were taken on each leaf. The length (in cm) of the primary rib ("PR_Length") and the length of the distance between the apex and the limit of the right leaf lobe ("Diagonal_Length") were measured using a meter. The secondary vein number of freshly collected leaves was also counted only on the side of the limb ("Rib_Number") (Annex 1).

Data analysis

The data treatments and analyses were made with different softwares. In a first time, the leaves' pictures were processed with the ImageJ sotfware version 1.47d (Wayne Rasband National institutes of Health, 2012) to make an Elliptic Fourier Analysis. Data were then analyzed using R software version 2.15.0 (R DEVELOPMENT CORE TEAM, 2012) and Past software version 2.17d (HAMMER *et al.*, 2001).

Table 1. Geographical details of sampled populations of *M. arborescens* (L.) Schott and *M. linifera* (Arruda) Schott, in French Guiana.

Population location name	Abbreviated pop. locality name	Species	Nb of Ind.	Coordinates
Kourou	Kou_A	*Montrichardia arborescens*	20	05°09'17,0" N, 052°41'19,6" W
	Kou_L	*Montrichardia linifera*	20	05°08'33,6" N, 052°40'10,4" W
Cayenne	Cay_A	*Montrichardia arborescens*	20	04°54'08,7" N, 052°18'09,0" W
	Cay_L	*Montrichardia linifera*	20	04°51'27,3" N, 052°15'48,0" W
Saint Laurent du Maroni	StL_A	*Montrichardia arborescens*	20	05°29'52,6", N 054°01'30,2" W
	StL_L	*Montrichardia linifera*	13	05°30'23,7" N, 054°01'23,1" W
Saint Georges	StG_A	*Montrichardia arborescens*	20	03°53'30,1" N, 051°48'24,2" W
Sinnamary	Sin_A	*Montrichardia arborescens*	20	05°22'37,5" N, 052°58'13,8" W
Maripa	Mar_L	*Montrichardia linifera*	17	03°48'06,7" N, 051°53'08,9" W
Kaw's swamp	Kaw_A	*Montrichardia arborescens*	20	04°29'46,2" N, 052°02'52,7" W
	Kaw_L	*Montrichardia linifera*	20	04°28'46,0" N, 052°03'25,4" W

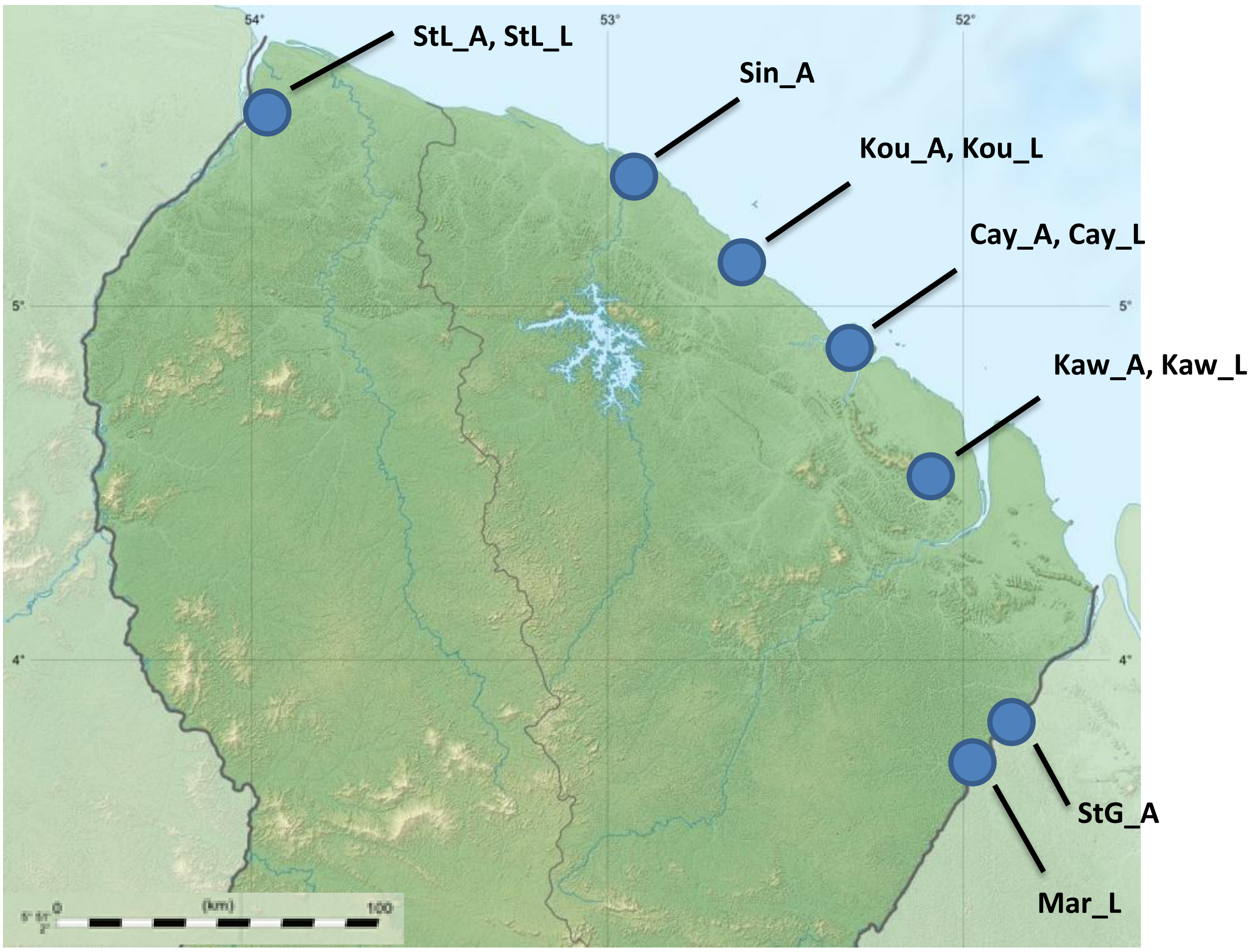

Fig. 1. Map representing the location of the populations of *Montrichardia* studied in French Guiana. cf. Table 1 for key to population codes.

Elliptic Fourier Analysis

The Elliptic Fourier Analysis (EFA) is used to describe outline shape and provide shape measures (ANDRADE *et al.*, 2008). EFA was carried out using the Elliptic Fourier Descriptor (EFD) plugin (BOUDIER & TUPPER, 2012; based on the descriptions taken from GLASBEY & HORGAN, 1995) with ImageJ software. The images were prepared for the EFA, with the same software. Each leaf was rotated so that the apex faced to the top with the midrib vertically. Paintbrush Tool was used to adjust the image to provide uniformity of tone along the contour; as well as minor damages of the outline were corrected.

The prepared images were re-opened in the software to execute the EFA program. In a first time, the image was changed with the “Make Binary” function, to obtain a black and white picture which can be analyzed with the EFD plugin. This EFD plugin was used to produce a normalized set of coefficients that are scale invariant (GLASBEY & HORGAN, 1995). The Fourier analysis was used with 42 descriptors, the number of descriptors used is arbitrary, resulting in a matrix of 168 descriptors summarize on one EFD coefficient column. The first two coefficients are ignored because they correspond to an arbitrary starting point, finally a set of 40 descriptors was obtained. To make easier the procedure, a macro was conceived to automatize the treatment of the 215 images. Finally the name of each result file was writed with the image number (Annex 2).

Finally, the EFD coefficients for all the leaves were gathered in one file for the subsequent statistical analyses.

Multivariate data analyses

First, the choice of the more effective combination of variables (morphological and/or morphometric) was made to avoid an excess of tests.

Then, the data analysis was splitted in three parts according to the types of tests. Thereby, Factorial Discriminant Analysis (FDA) was used on the data to discriminate the two species on a regional scale, the sites and then species by species among the sites.

Afterward, a NPManova was performed on the whole data to compare the regional scale to the local scale. This non-parametric analogue of MANOVA which calculates a F value with significance values computed from 10000 replicate permutations of group membership. This test allows to see if there are any differences between the species in each site, and hence if the two species of *Montrichardia* can be differentiated from a same place.

Finally, the third test was a Linear Discriminant Analysis (LDA) which is used to do predictions in order to verify the quality of the previous ranking of the populations.

Results

Preliminary analysis

Three types of variable combinations could be used for the different comparisons: Morphological variables, Morphometric variables or both. The Factorial Discriminant Analysis (FDA) made on the 40 shape variables of the elliptic Fourier analysis and the result of the NPManova, both made with all the data sets, indicate that there is a better differentiation combining both types of variables. For this reason the following comparisons are made with both the morphological and the morphometric variables.

Comparison of the two species

The variables of the Factorial Discriminant Analysis (FDA, Fig. 2) are dispatched on two axis, axis 1 represents 20.73 % of the total information and the second axis represent 17.06%. It should be noted that all FDA present in this study were validated by a Monte Carlo permutation test (p-value: $9.99*10^{-4}$), this means that FDA allowed a good discrimination of the considered groups (populations, sites or species). It can be observed that there are differences between the two studied species of *Montrichardia* among the first axis (Fig. 2b). There is an efficient separation of the species although there is a certain overlap in the distribution of the individuals. The rib number, the diagonal length and the EFD_2 seem to explain some information on axis 1 but these variables are more represented on axis 2. In fact the primary rib length has a strong weight and explains more on the first axis. So the two species can be differentiated under a gradient where *M. linifera* represents the large primary rib lengths (23.22 ± 4.86) and *M. arborescens* the shorter ones (16.1 ± 2.8).

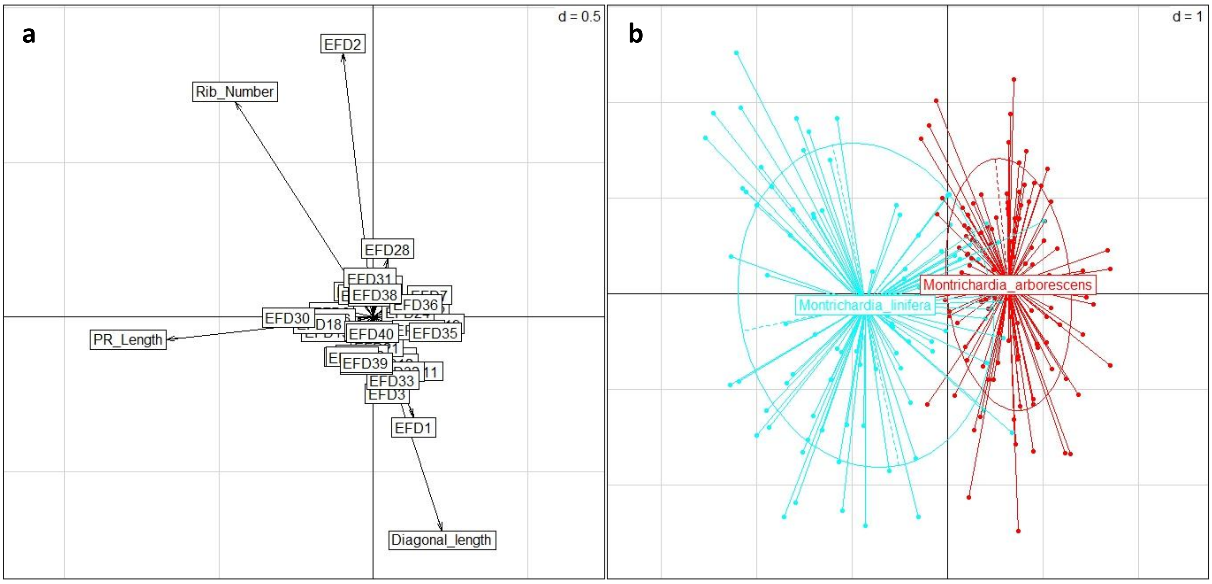

Fig. 2. Factorial Discriminant Analysis of the two *Montrichardia* species. **a.** Factorial maps for the projection of the vectors based on the canonical weights of the first two principal components of the FDA (37.79% of the variance explained) made from 40 shapes variables and the five morphological variables. This plot is applicable on Fig. 3 too. **b.** Factorial maps with representation of point classes according to the canonical scores of the FDA.

Comparison between sites

This test is used to see how the measured variables varied among the sites. This comparison shows that there are differences among the sites. Indeed, Kaw is the site which has the large rib number and the highest EFD_2 in both species (Fig. 2a, Fig. 3). Kourou seems to be the opposite, and the other sites do not differ. A particular strong variability occurs in Cayenne due to variable primary rib lengths whereas in St Laurent the high variability related to any particular variable.

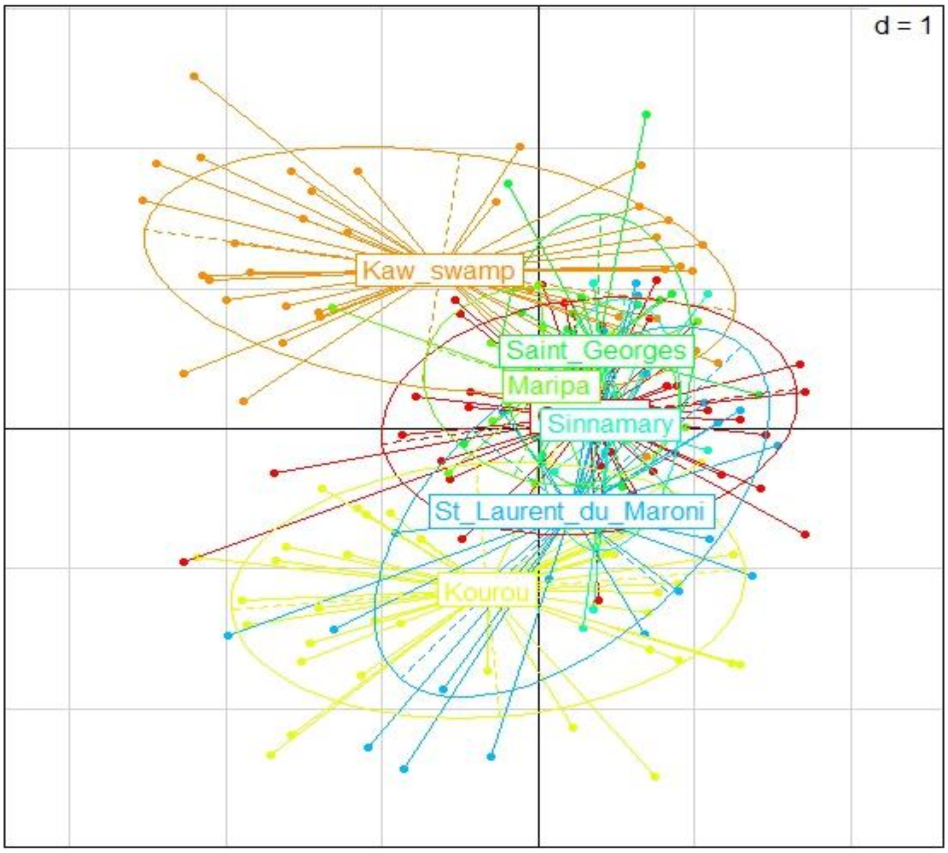

Fig. 3. Factorial Discriminant Analysis of the sampled populations. Plot of factorial maps with representation of point classes populations with two principal components of the FDA test. It represents the disposition of the individuals of both species within different sites according to the canonical scores of the principal component.

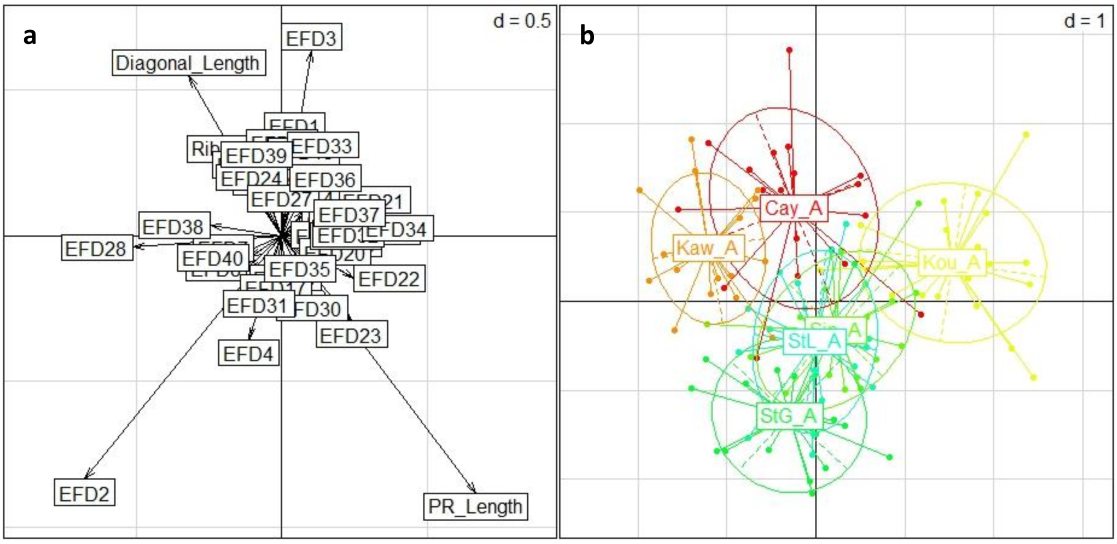

Fig. 4. Factorial Discriminant Analysis of *Montrichardia arborescens* population. **a.** Factorial maps for the projection of a vector basis based on the canonical weights of the first two principal components of the FDA made from 40 shapes variables and the five morphological variables. **b.** Factorial maps with representation of point classes according to the canonical scores of the FDA.

Interpopulational comparison in *M. arborescens*

In the following FDA (Fig. 4), the information explained by the first axis and the second axis are respectively 27.71% and 24.80%.

Populations of *M. arborescens* of Kaw and Cayenne have the highest diagonal length and the smallest primary rib length (Fig. 4). St Georges had the highest EFD_2 and a smaller diagonal length and EFD_3. Moreover, it appears that Kourou got the smallest EFD_{28} of all the populations, and that St Laurent and Sinnamary populations have rather similar leaves.

Interpopulation comparison in *M. linifera*

In the *M. linifera* FDA (Fig. 5), the information explained by the first axis and the second axis are respectively 31.18% and 28.36%.

The populations are well separated from each other in the FDA and *M. linifera* presents a large range of variability (Fig. 5b). Indeed, Kourou and St Laurent have the highest diagonal length and primary rib length whereas Maripa and Cayenne have the smallest. Kaw have the highest number of ribs, and Saint Laurent the fewest.

Interpopulational and interspecies comparison

The NPManova test enabled to confirm that in each site, there is a significant difference between the two species, and between the different populations of *M. linifera*. However, this is not the case for *M. arborescens* because many populations appeared to be similar (cf. Table 2).

Verification by LDA

The first LDA test is based on the sites comparisons and showed that only 102 of 210 (48.6%) individuals were correctly assigned. The second LDA was made from the comparison of the two species and mentioned that 81.9% of the individuals are placed in the corresponding species.

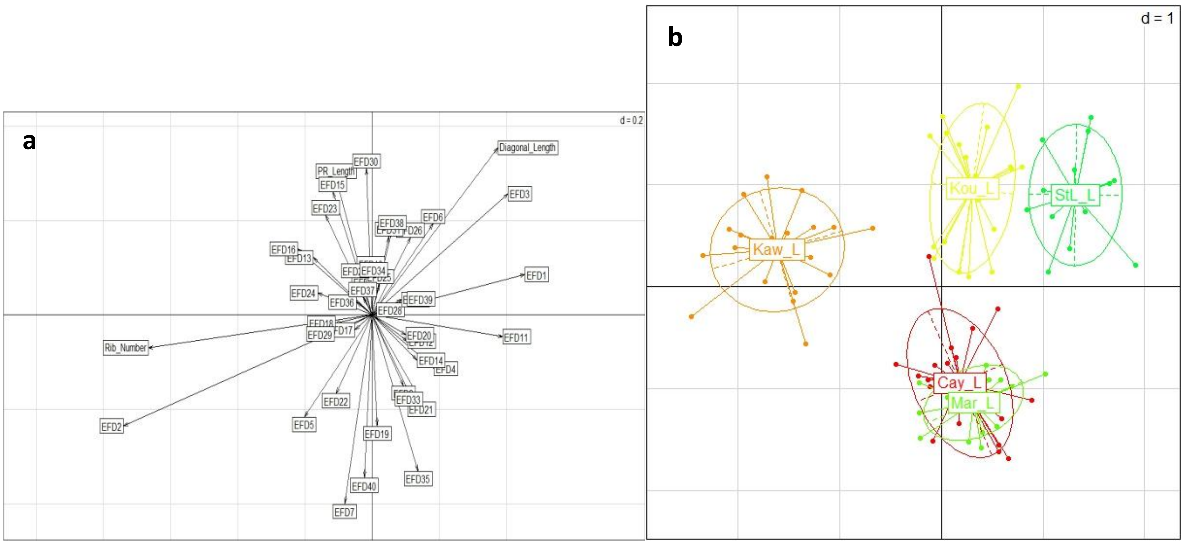

Fig. 5. Factorial Discriminant Analysis of *Montrichardia linifera* population: Factorial maps for the projection of a vector basis based on the canonical weights of the first two principal components of the FDA made from 40 shapes variables and the five morphological variables. **b.** Factorial maps with representation of point classes according to the canonical scores of the FDA.

Table 2. Differences between eleven populations of *Montrichardia*. Values shown are p-values (probability that two groups are the same) derived from NPMANOVA. cf. Table 1 for key to population codes. ns : not significant.

	Kou_A	Kou_L	Sin_A	StL_A	StL_L	Cay_L	Cay_A	Mar_L	StG_A	Kaw_L
Kou_L	0.0001	-								
Sin_A	ns	0.0001	-							
StL_A	ns	0.0001	ns	-						
StL_L	0.0001	ns	0.0001	0.0001	-					
Cay_L	ns	0.0001	0.0001	0.0017	ns	-				
Cay_A	0.0001	0.0001	0.0003	0.0001	0.0001	0.0001	-			
Mar_L	ns	0.0001	ns	ns	0.0001	ns	0.0001	-		
StG_A	ns	0.0001	ns	ns	0.0001	0.0004	0.0007	ns	-	
Kaw_L	0.0001	0.0002	0.0001	0.0001	ns	ns	0.0001	0.0001	0.0001	-
Kaw_A	0.0002	0.0001	ns	0.0018	0.0001	0.0001	ns	ns	ns	0.0001

Discussion

In this study, about the characterization of leaf morphology in two species of *Montrichardia*, 210 leaves were analyzed. The variation of leaf morphology was studied at two levels, interspecific and interpopulation.

Interspecific level

At the interspecific level, the differentiation between the two species is more supported by the morphological traits, particularly by the primary rib length which represents most of the discriminant information. This seems to be consistent with the direct observations (Annex 3 & 4). Furthermore, it's well known that *M. linifera* presents a larger leaf and has more rib than *M. arborescens* (CROAT *et al*., 2005) but no quantitative study was available. Therefore, the results show that *M. linifera* has a significant higher length of the primary rib than *M. arborescens*.

The overlap observe in the interspecific comparison could be hypothetically imputed to a presence of hybrid in the individuals' sampled.

Inter-sites level

The analysis on the different studied sites pooling the two species, highlights that Kourou and Kaw's populations are distinct from the others. This result is mainly due to the fact that *M. linifera* and *M. arborescens* have more ribs, a greater EFD_2, and a low diagonal length in Kaw's site and the opposite in Kourou's site. These differences could be explained by environmental differences (not measured in this study) between these two sites. A particular interest is the fact that Kaw is a swamp habitat whereas Kourou is a river flooded area, the other sites were collected along river or stream banks.

Interpopulational level

The results of the comparison of the different *M. linifera*'s populations show that unlike to previous results, the morphometric aspect is more involved in the discrimination of these populations. The overlap of the two populations of Cayenne and Maripa could reflect the fact that they have similar foliar characteristics. The Kaw's population of *M. linifera* has the highest rib's number. According to SILVA *et al*. (2012), there is possibly a covariation between cordate leaf blades and a higher number of secondary veins in this species.

In the case of *M. arborescens*, the populations are less differentiated by the morphometric aspect than for *M. linifera*. Even if the populations are well separated by the EFD_2 and the EFD_3, the part of leaf these descriptors represents can't be known because the EFA is a quantitative test, so to use the EFD is necessary to have significant most of them. Therefore, the distinction between the populations of the two species could be interpreted by the fact that the *M. arborescens*'s leaf shape seems to be more differentiated by morphological variables than the morphometric variables, and the opposite for *M. linifera*'s leaf shape.

According to the words of BARBOSA *et al.* (2004) who said that the sampled habitats could be considered as unique assemblages of taxa with distinct histories. Therefore, these populations could differ due to their own history, for example because of the colonization of different types of habitats, especially among a flood gradient of the individuals.

Interpopulational and interspecies comparison

The NPManova highlights that even if there are different species populations with similar leaves, there is a significant dissimilarity between the two species within each site.

Verification by LDA test.

The LDA test permited to show that the distinction of the species was correct. Like the previous results, the LDA shows that there is an efficient discrimination when only the two species were compared and not when the comparison was made among the populations.

Conclusion

This study shows that at the interspecific level, morphological variables gave a better differentiation resolution than the morphometric ones. On the other hand, at the intraspecific level, the differentiation among populations was due to different variable sets according to the species. In *M. arborescens*, morphological characters were more pertinent in differentiating the populations, whereas in *M. linifera* morphological characters were also differentiating the populations but morphometrics ones were also very informative but their relative importance was not assessed.

Even if the multivariate analyses were able to differentiate the two species within each studied site, one population from one species could be similar to another population from the other species. This result suggests that the variation of leaf studied traits was as important within site between the two species as among sites within a given species.

One limit of this study was that the leaf characters used were mainly pertinent to differentiate the two species but not so good to discriminate the different populations. Other characters should be added in order to increase population discrimination.

In perspective, this preliminary study could be improved by the sampling by collecting along transects on each study site in order to cover a wider range of variability of the population and affine the treatment of the EFD, or using landmarks.

Another study would be to analyze the variation of these two species and eleven populations with genetic tools through the development of microsatellites marker. It's could be interesting to underscore the distinction of species and confirm or not the presence of hybrids (interspecific polymorphism) and the study of the diversification and structuration of the populations of *Montrichardia* (intraspecific polymorphism), as it has been done on other Araceae (ANDRADE *et al.*, 2008; 2010).

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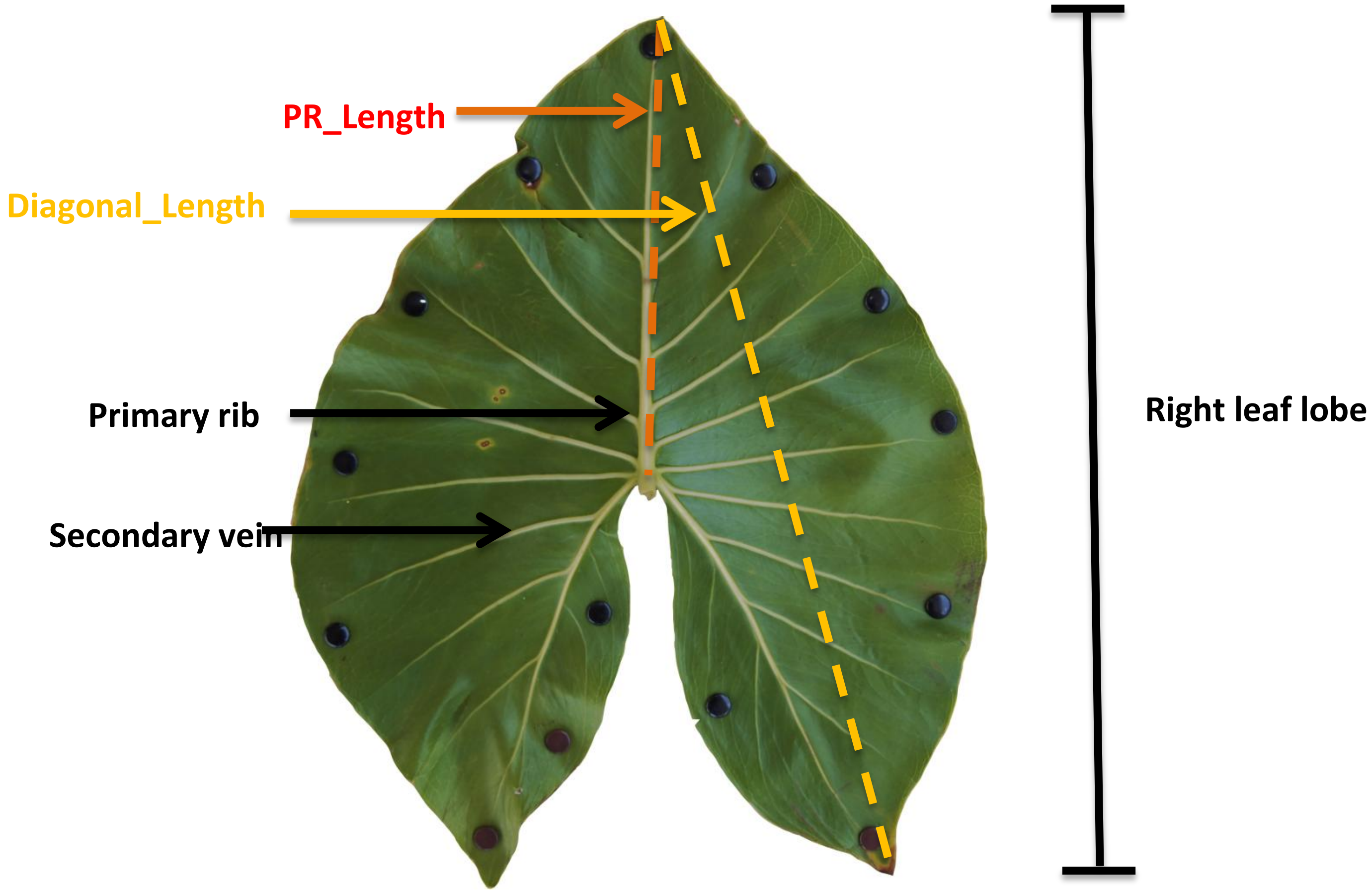
ANNEX 1



Picture 1. Leaf blade of Montrichardia. PR_Length : length (in cm) of the primary rib. Diagonal_Length : length of the distance between the apex and the tip of the right leaf lobe.

ANNEX 2

##Select the first image ("i=0") to the last image of the population ("i = 20"), with a step of 1.

```
for(i=0;i<=20;i+=1) {
```

##Make a black and white image then make an automatic threshold.

```
run("Make Binary");
```

##Make an analyze of the particles with the settings given: the size (from 1000000 pixels to infinite to select the leaf image), and the circularity (no selection here).

```
run("Analyze Particles...", "size=1000000-Infinity circularity=0.00-1.00 show=Nothing display clear include add");
```

#Selects all the previous objects and assigns them a number.

```
roiManager("Show All with labels");
```

#Selects the first object (the bigger, so the leaf).

```
roiManager("Show All");
roiManager("Select", 0);
```

#Make the Fourier analysis on the selection with 42 harmonics and EFD.

```
run("EllipticFD ", "number=42 results reconstruction");
```

#Allows the save of the test results with opening the saving window.

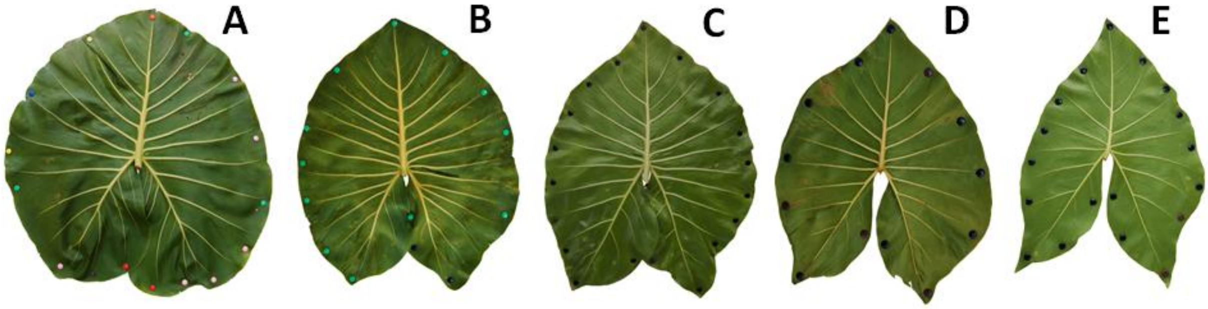
```
saveAs("Results");
```

#(The last four) Clear the results and close all the useless windows.

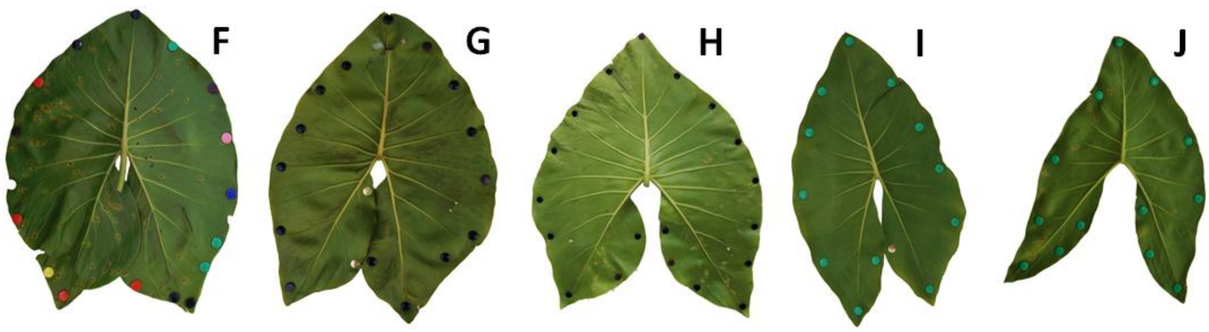
```
run("Close");
close();roiManager("Delete");
IJ.deleteRows(0, 0);
run("Close");
```

Annex 2 : Macro for the extraction of the EFD. Used with the ImageJ sotfware version 1.47d (Wayne Rasband National institutes of Health, 2012)

ANNEX 3

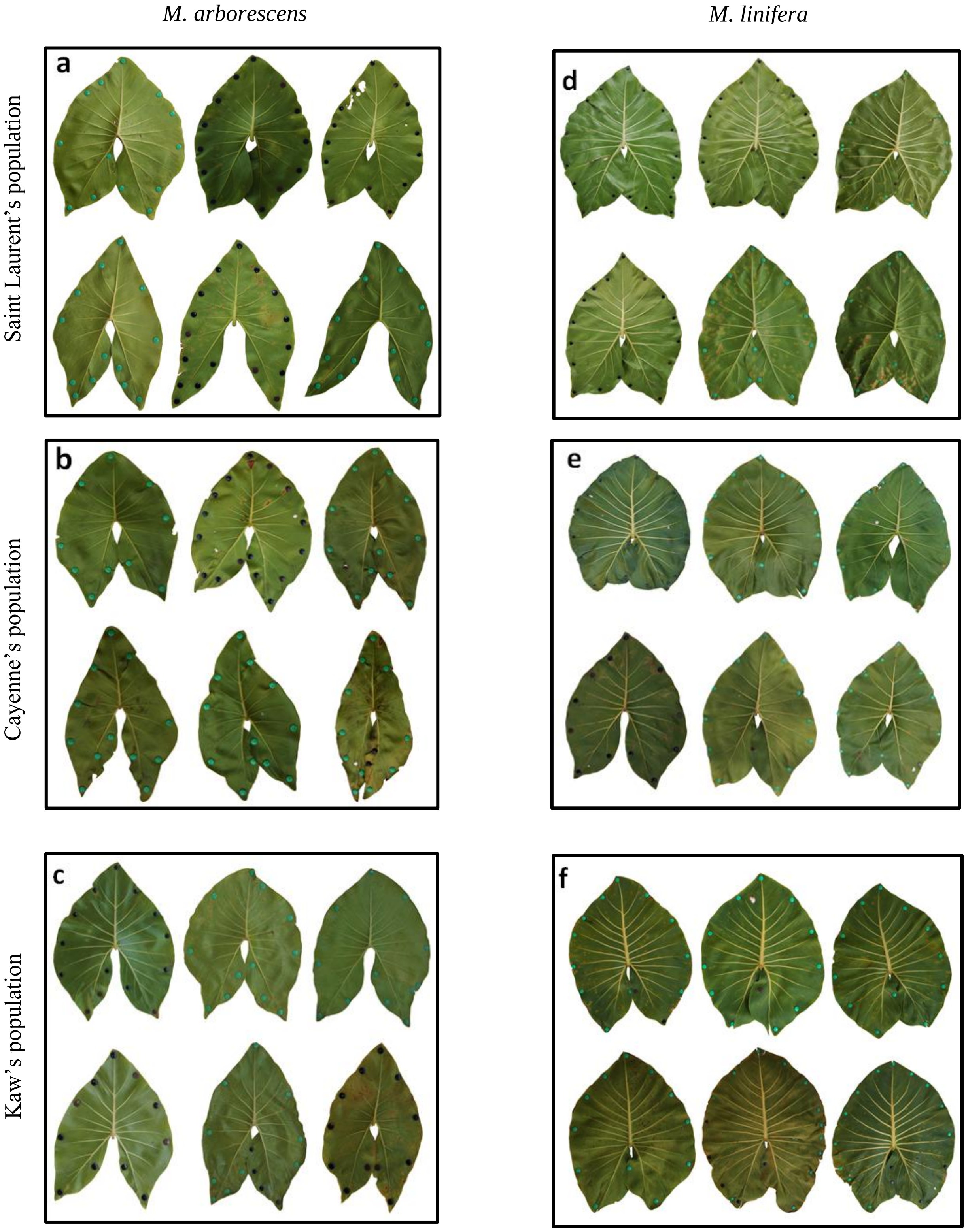


Pictures 2. Leaf shape variation of different populations of *M. linifera*: The leaf A comes from the Kourou's population, the B from the population of the Kaw's swamp, the leaves C and E were collected from a Saint-Laurent population and the leaf D correspond to the Cayenne population.



Picture 3. Leaf shape variation of different populations of *M. arborescens*: The leaf F corresponds to the Kourou's population and the leaf G to the population of Cayenne. The leaf H comes from the Kaw swamp population and J was collected from a Saint Laurent's population and the leaf I from the population of Saint Georges.

ANNEX 4



Picture 4. Interpopulational shape variations in *M. arborescens* (a, b, c) and *M. linifera* (d, e, f) for three populations: St Laurent, Cayenne and Kaw's populations.