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UNDERSTANDING DIVERSITY IN LEAF SHAPE OF CHINESE *SAGITTARIA* (ALISMATACEAE) BY GEOMETRIC TOOLS

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Abstract

Plants of *Sagittaria* generally possess different leaf shapes, but these shapes were rarely quantified. In this study, all species of Chinese *Sagittaria* were collected, and their leaf shapes were analyzed using two geometric tools: Topological description and geometric morphometric analysis. The leaf shapes of Chinese *Sagittaria* can be divided into three major groups according to topological types of the venation architecture, and the variation of leaf shape is closely related to the development degree of *Sagittaria* plants. Even if corresponding to the same venation pattern, many leaf shapes have evident differences which could be revealed by geometric morphometric analysis, and these differences show species specificity to a great extent. Making use of these results, some misunderstandings in the taxonomy of *Sagittaria* also be cleared up.

Key words: Geometric morphometric analysis, Leaf shape, *Sagittaria*, Topological description.

Introduction

As a major photosynthesis organ, the leaf is extremely important to the survival of plants. In different plants, it may appear much diversified in size, shape, color and spatial array, etc. Among these characters, the shapes of leaf have drawn much attention by many investigators all the time, especially the plant physiologists and taxonomists (Thompson, 1917; Ashby, 1948; Gleissberg & Kadereit, 1999; Bell & Bryan, 2008; Nicotra *et al*., 2011; Moosavi, 2012; Amanullah *et al*., 2013).

Sagittaria (Alismataceae) is a worldwide aquatic genus, and occur in extensive habitats (e.g., marsh, canal, pond and paddy field), so shapes of their leaves are very flexible (Wooten, 1973, 1986; Chen, 1989). Some species may have several types of leaves (e.g. *Sagittaria. sagittifolia*), and their shape vary remarkably (Hroudov, 1980). Even if being the same type, the leaves may emerge different patterns according to various water levels, soil nutrient and other some environmental factors (Wooten, 1986; Dorken & Barrett, 2004; Richards & Ivey, 2004).

According to previous research, the diversity of *Sagittaria* leaf has been widely recognized (Wooten, 1970, 1973). And leaf shape plays an important role in taxonomy of this genus, for instance, whether the mature leaves are sagittate or the ratio of leaf length to leaf width and (Chen, 1989). However, most of the studies on leaf shape only remain in the descriptive stage (Adams & Godfrey, 1961; Wooten, 1973; Bloedel & Hirsch, 1979; Ozen, 2012). As the limitation of traditional morphological description, it is easy to make mistakes in phylogenetic classification, and some relationship between phenotypes is difficult to be found (Rataj, 1972; Chen, 1989). Recently, new geometric methods are led into morphology, and it has been proved successful to a great extent in taxonomy (Adams *et al*., 2004; Shipunov & Bateman, 2005; Viscosi, 2009; Viscosi & Cardini, 2011). In this study, we try to analyze the leaf shapes of *Sagittaria* in China by these geometric tools. Here, we hope to solve: (1) How to quantify the leaf shapes of in *Sagittaria*? (2) What are the difference and connection between these leaf shapes? (3) Is there evident interspecific difference between the leaf shapes of Chinese *Sagittaria*?

Materials and Methods

Sagittaria in China includes *S. guayanensis*, *S. tengtsungensis*, *S. lichuanensis*, *S. potamogetifolia*, *S. pygmaea*, *S. natans* and *S. trifolia* along with *S. trifolia* var. *longiloba* and *S. trifolia* var. *sinensis* (two varieties). We collected hundreds of samples of them during 2008-2012, and cultured in the greenhouse at Wuhan University.

Morphological analysis: Morphological analyses were performed by means of two different geometric methods: topological description of leaf venation patterns and geometric morphometric analysis of leaf shape based on landmarks.

Topological description was carried on living plants cultured in our greenhouse. We respectively chose 5 plants of each species, and then observed their leaf sequences from germination to blossom. The shapes and venation structures of all leaves in these sequences were recorded by an Olympus EVIL camera. In the leaf venation architecture of *Sagittaria*, only major longitudinal veins were considered, which were indicated by a series of successive line in a graph, and the nodes were employed to show junctions of these veins. Through this treatment, venation architecture of each leaf corresponded to a topological graph. The detail topological method in our study referred to the report of Nebelsick *et al*., (2001).

Geometric morphometric analysis was only used in the leaf shapes of S. *guayanensis*, S. *lichuanensis*, S. *potamogetifolia*, S. *natans*, S. *trifolia* and its two varieties during florescence, because these leaf shapes were complex and had same homologous points. 700 individuals (every species with 100 ones) with at least a complete leaf and reproductive structure were selected from 3000 specimens from our greenhouse or Wuhan University Herbarium, whose collecting locations of these specimens was shown in Fig. 1. For each selected specimen, we chose one leaf and obtained its leaf photograph by a scanner (Cannon 8400F). Based on these photos, two data sets were built: the first one contained leaf images of all above species and varieties; and the second set was only made up of photos of S. *lichuanensis*, S. *potamogetifolia*, S. *trifolia* and its two varieties. These data sets were used to obtain 12 landmarks of leaf as in Fig. 2 via TPS software, of which 6 odd landmarks were chosen following Dorken *et al*., (2004), and remaining even landmarks were inserted median points by which more shape information could be obtained.

Data analysis: For the landmark data of leaf shapes, we performed a generalized Procrustes analysis (GPA) to extract shape and size components of form variation respectively (Viscosi & Cardini, 2011). Using the variance-covariance matrix of the GPA shape coordinates, we carried out a Principal Component Analysis (PCA) on the first data set, a Canonical Variate Analysis (CVA) on the second data set and a Discriminant Function Analysis (DA) with pairwise computation of *S. potamogetifolia* and *S. trifolia* using leaf shape, and a Procrustes ANOVA testing shape variation at two levels: intraspecies and interspecies. Most of the analyses were accomplished in two freeware MorphoJ and Past.

Results

Topological description: The leaf shape of *Sagittaria* plants has a close relationship to the leaf venation. According to the topological description, there are 3 categories as in Fig. 3 in the leaf venation architecture of Chinese *Sagittaria*, and all leaf shapes can be divided into three major groups according to these types: group A contains strip-shape corresponding to type A venation with only one junction; group B consists of spear-shape and spoon-shape to type B with two junctions; group C contains heart-shape, boat-shape and arrow-shape to type C with four junctions. In addition, during the development of plants of *S. tengtsungensis* and *S. pygmaea*, only type A and type B venation could occur. However, in other Chinese *Sagittaria*, the leaf shapes belong to above three groups are able to appear in fixed order as growing (group A→group B→group C), and the venation of their mature leaves emerging in florescence are all type C.

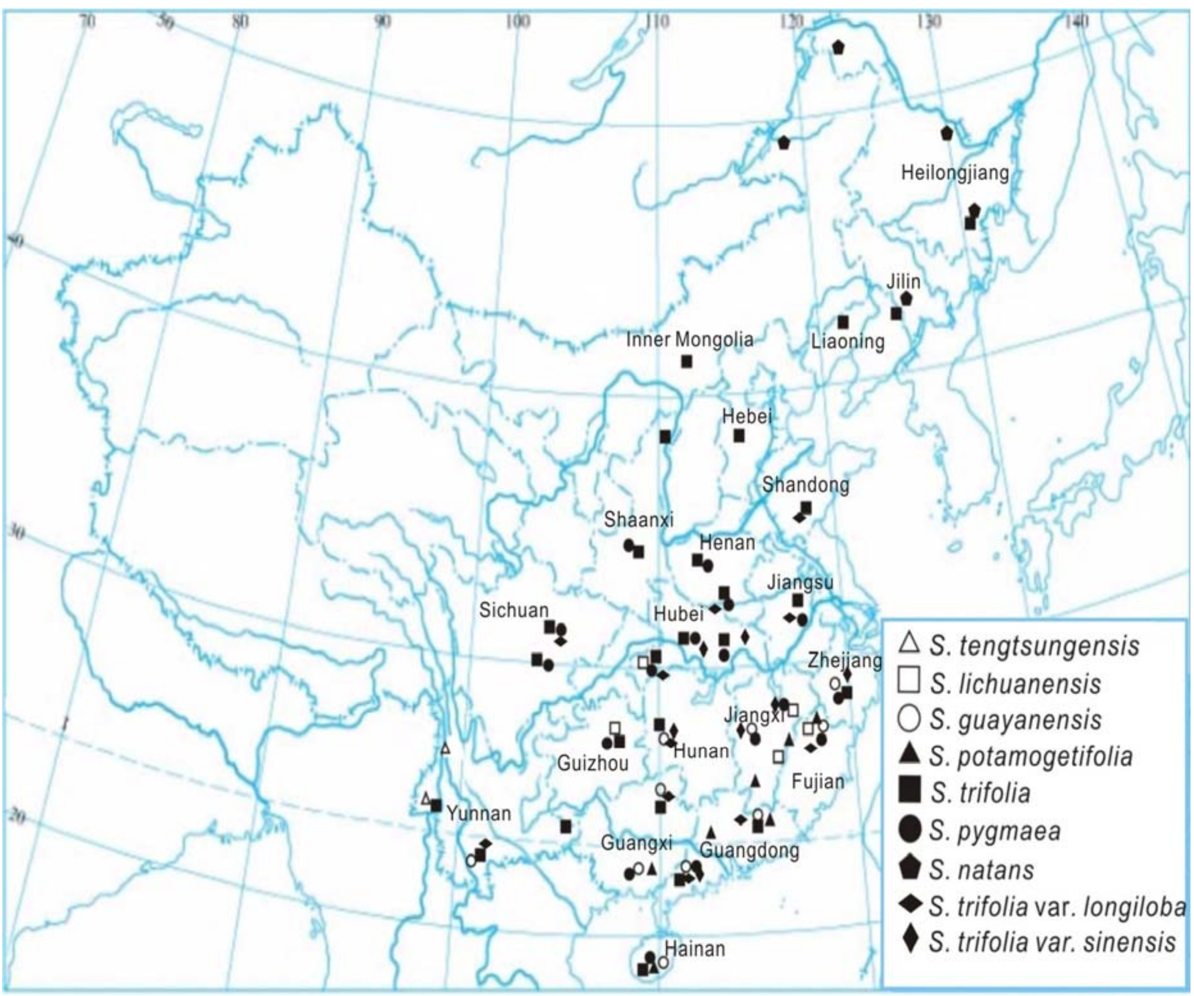

Fig. 1. The collecting locations of all specimens in this study.

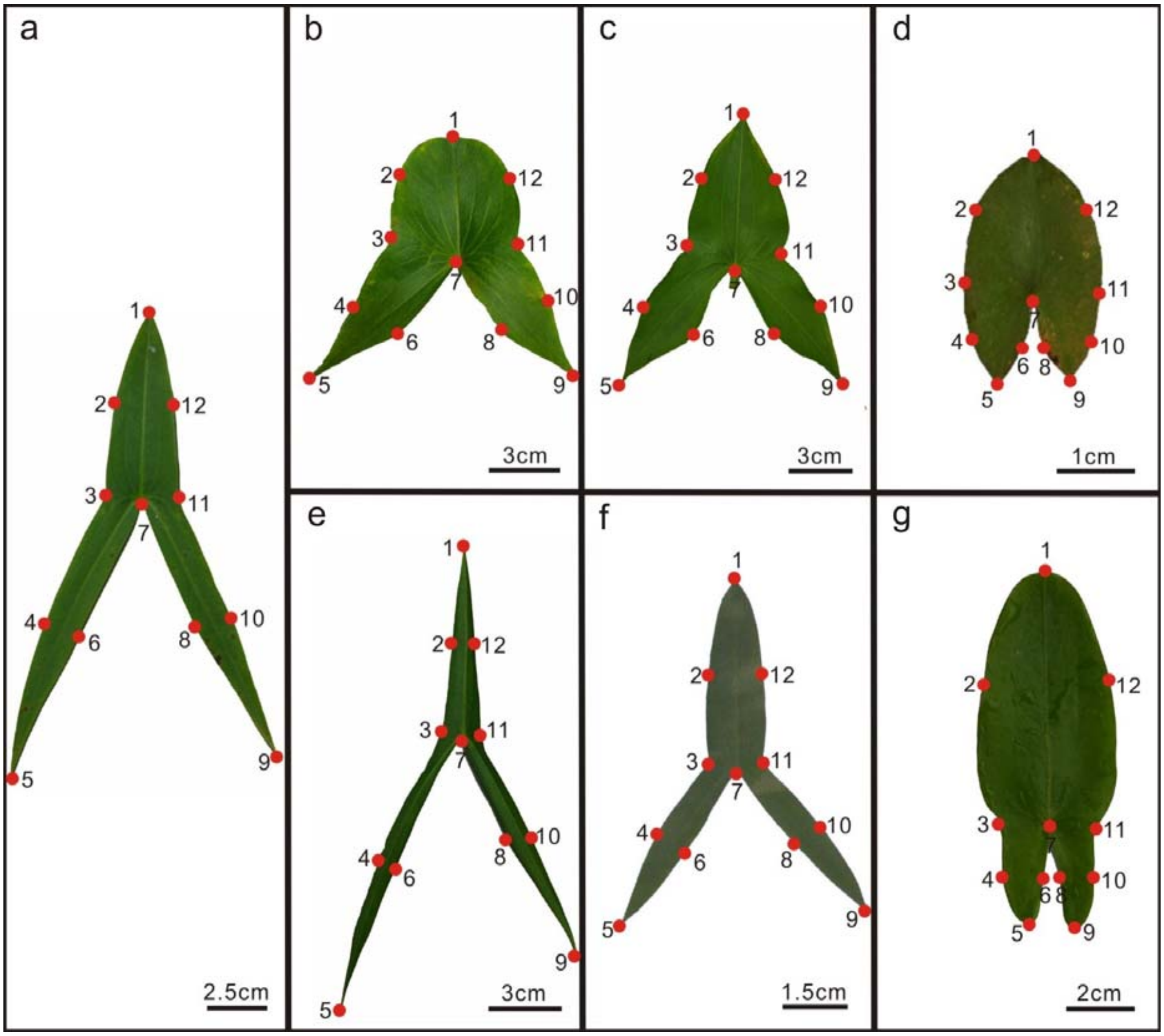

Fig. 2. The example of leaf with 12 landmarks for each species studied in geometric morphometric analysis. (a) *S. trifolia*; (b) *S. trifolia* var. *sinensis*; (c) *S. lichuanensis*; (d) *S. guayanensis*; (e) *S. trifolia* var. *longiloba*; (f) *S. potamogetifolia*; (g) *S. natans*. Red dots represent landmarks. Odd landmarks were chosen following Dorken *et al*., and even landmarks were inserted median points, each of which is the same distance from neighboring two odd landmarks.

Geometric morphometric analysis: PCA on the standardized landmark-coordinates matrix of the first data set reveal three most important principal components including 94.1% of the total variance. The pairwise plot of PC1 and PC2 return much taxonomic structure, which separate *S. guayanensis*, *S. natans* and *S. Lichuanensis* from other species clearly, and discriminate *S. trifolia* var. *longiloba* and *S. trifolia* var. *sinensis*, but *S. potamogetifolia* and *S. trifolia* nearly mix together. The appending of PC3 does not make the above condition better, so we only show the scatterplot of PC1 vs PC2, with the leaf shapes corresponding to the two extreme values of the distribution of each relative warp (Fig. 4). In MorphoJ, using a Procrustes ANOVA, we respectively set species as main effect and leaves as a random effect. The analysis results for shape variation are shown in Tables 1-2. Species explain about 75% of total sum of squares, and this effect is statistically significant ($p<0.0001$). These results indicate that there are interspecific differences between the leaf shapes in the first data set.

From the CVA results of the second data set, we obtain a scatter diagram (Fig. 5a). In this diagram, although the five groups of points represent the leaf shapes of *S. lichuanensis*, *S. potamogetifolia*, S. *trifolia* and its two varieties all concentrate on a large middle area, there is only few overlap among them except that the points of *S. trifolia* are difficult to be distinguished from its two varieties. As in the CVA, the results of the DA (Fig. 5b) on the pair of *S. potamogetifolia* and *S. trifolia* indicate the range of leaf shapes rarely overlapped between these two species. Similarly, a Procrustes ANOVA on the leaf shapes of S. *potamogetifolia* and S. *trifolia* demonstrates that for shape variation the most of the total sum of squares comes from the leaves rather than species, but the main effect of species is still statistically significant ($p<0.0001$), which indicate that although there are large intraspecific variation in the leaf shapes of *S. potamogetifolia* and *S. trifolia*, the difference of leaf shapes between these two species is obvious.

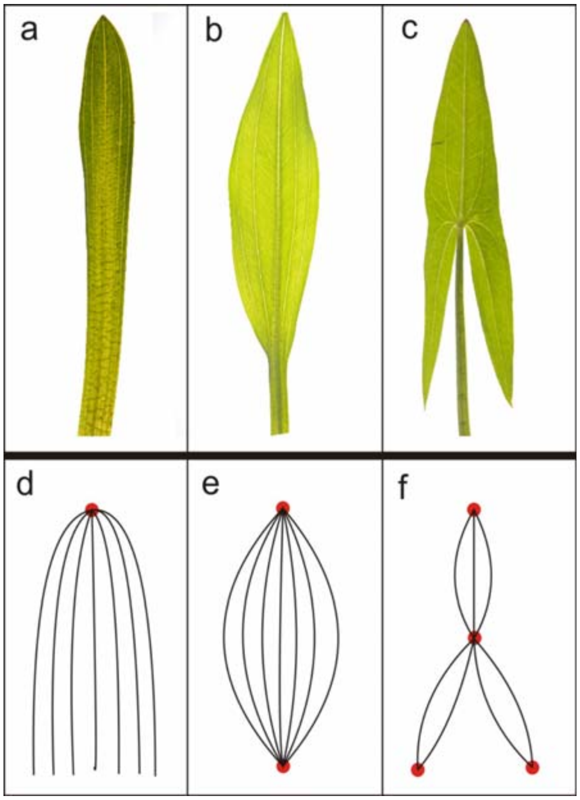

Fig. 3. Leaf venation architecture of *Sagittaria*. (a-c) the representation of three kinds of leaf shapes. (a) Strip-shape, (b) spear-shape, (c) arrow-shaped. (d-f) the topological graphs of three types of venation architectures respectively corresponding to above three leaf shapes, in which the large longitudinal veins are expressed as some successive lines and the nodes indicate the junctions of these veins. (d) Type A to strip-shape, (e) type B to spear-shape, (f) type C to arrow-shape.

Table 1. Procrustes ANOVA of shape variation in the first data set.

Effect	SS	MS	df	F	P
Species	31.866	0.265547	120	313.92	<0.0001
Leaves	11.724	0.000846	13860	NaN	NaN

Table 2. Procrustes ANOVA of shape variation between *S. potamogetifolia* and *S. trifolia*.

Effect	SS	MS	df	F	P
Species	0.599	0.029975	20	30.06	<0.0001
Leaves	3.949	0.000997	3960	NaN	NaN

Discussion

Previous research has shown that the leaves of *Sagittaria* possess more than five kinds of shapes and heterophylly occurs widely in this genus (Bogin, 1955; Wooten, 1973). The ontogenetic sequence of the heteroblastic type is regarded as the cause of leaf variations in *Sagittaria* (Wooten, 1986). In our study, different shape and venation structure of leaves is observed to occur with the development of the Sagittaria individual. There is an intimate relationship between leaf shape and vein pattern, and the pattern of the major veins reflects leaf shape (Nelson & Dengler, 1997; Dengler & Kang, 2001). The venation pattern in Chinese *Sagittaria* can be divided into three types by topological description, and these three types only can occur in a fixed order in ontogenesis (type A→type B→type C). Considering that venation architecture impacts photosynthesis via its effect on water transport (Brodribb *et al*., 2007), the emergence of different venation pattern and corresponding leaf shape could have important physiological significance to the growth of *Sagittaria*.

From germination to blossom, the leaf venation architecture of many species in Chinese *Sagittaria* undergoes a same transformation, but in the growth of *S. tengtsungensis* and *S. pygmaea,* type C venation does not appear, which may be the result of some special developmental regulation. Differing from type A or type B, the topological graph of type C venation has a complex structure, and this complexity endows the corresponding leaf shape with great variability. However, all leaf shapes corresponding to same type venation have same homologous points, and they could be analyzed together by geometric morphometric tool (Zelditch *et al*., 2004).

The mature leaves of *S. guayanensis* and *S. natans* are respectively heart-shaped and boat-shaped, and they are generally classed as nosagittate leaf (Bogin, 1955). But the leaf venation architecture of them is same as sagittate leaf. Differing from other species, *S.guayanensis* and *S.natans* are floating palnts, whose mature leaves float on water (Chen, 1989). In the PCA of first data set, the leaves of *S. guayanensis* and *S. natans* could be distinguished from other species by PC2, and they have approximate PC2 scores. To much extent, the relative warp corresponding to PC2 represents the change of leaf shape from emergent to floating. In addition, although all being floating plants, *S.guayanensis* and *S.natans* respectively distribute in different areas: the former grows in the tropic and the latter grows near to the cold zone. From the PCA scatter diagram, we also find the evident difference of leaf shape of these two species, in which PC1 involving the ratio of leaf length to leaf width plays a very important role.

All species of Chinese *sagittaria* with sagittate leaves are emergent plants, in which *S. lichuanensis* belongs to subgenus *Lophotocarpus*; and *S. potamogetifolia*, *S. trifolia* belong to subgenus *Sagittaria* (Chen, 1989). Nearly all scatter represent leaf shapes of them lie in the lower half of PCA diagram, and *S. lichuanensis*'s scatter concentrate in a small area different from other species. *S. lichuanensis* and *S. trifolia* var. *sinensis* all have broad mature leaves (large ratio of leaf width to leaf length), and it is difficult to discriminate between them only by naked eye. However, in PCA diagram, there is few overlap between these two species' leaf shapes can be found, which means their leaf shapes still have essential difference. Compared to the stable leaf shape of *S. lichuanensis*, the leaves of *S. trifolia* and its two varieties have diversified pattern. *S. trifolia* var. *longiloba* generally grows in some wet land of mountains, and possesses long and narrow leaves; *S. trifolia* var. *sinensis* is artificially cultivated, and its leaves is relatively broad. In these two varieties, there are some leaves share the same form with some leaves of *S. trifolia*.

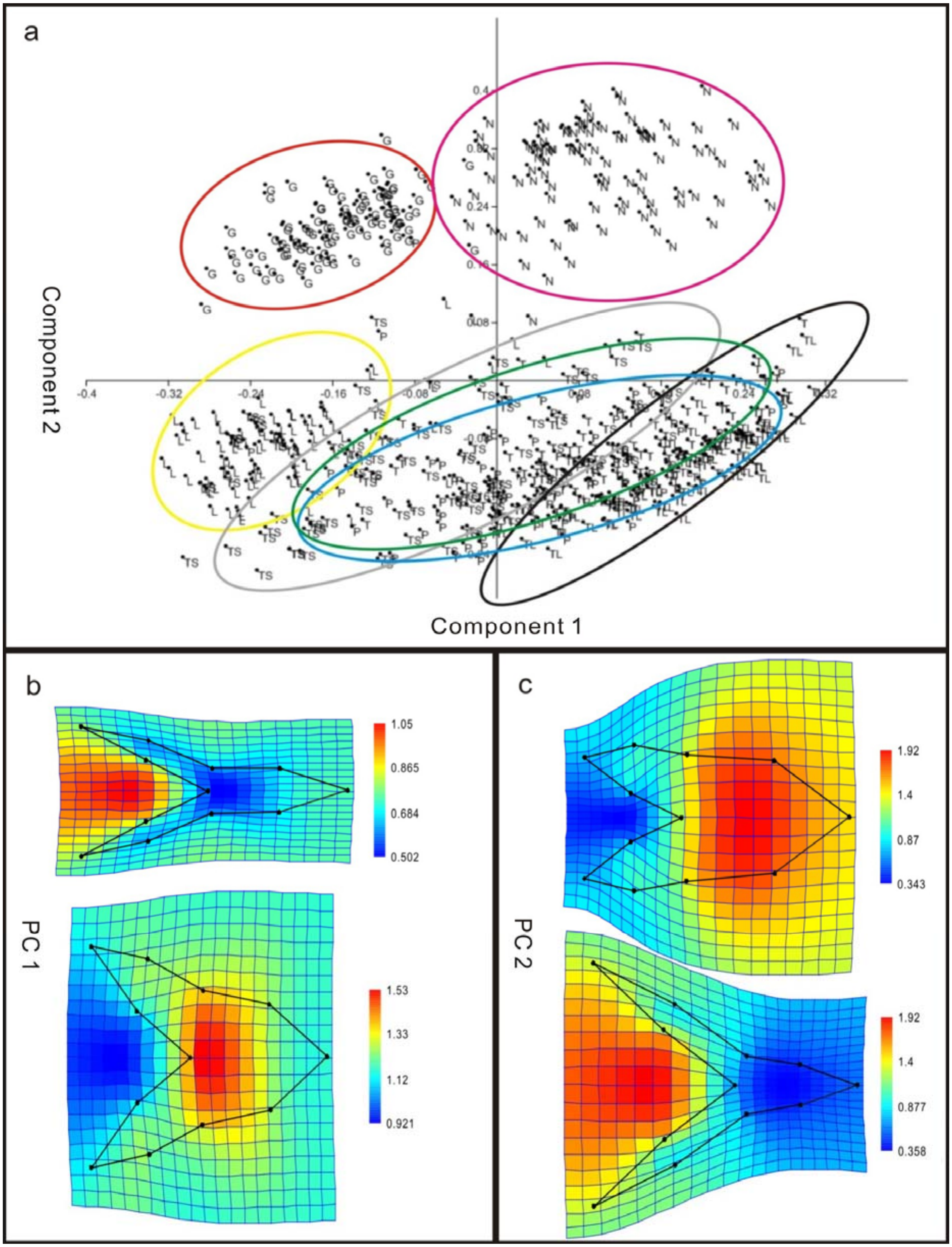

Fig. 4. Geometric morphometric analysis of the first data set including the leaf shapes of five species and two varieties with type C leaf venation. (a) Scatterplot of PC1 vs PC2 in PCA. Color ellipses on the picture consist of 95% scatter points corresponding to each species, red ellipse is related to *S. guayanensis*, pink to *S. natans*, yellow to *S. lichuanensis*, gray to *S. trifolia* var. *sinensis*, green to *S. trifolia*, blue to *S. potamogetifolia* and black to *S. trifolia* var. *longiloba*. (b-c) leaf shapes corresponding to the two extreme values (1 upper and -1 lower) of the distribution of two principal components: (b) PC1; (c) PC2. The colour coded Jacobian expansion factors are used to measure the degree of local expansion or contraction of the grid: yellow to orange red for factors>1, indicating expansions; light to dark blue for factors<1, indicating contractions.

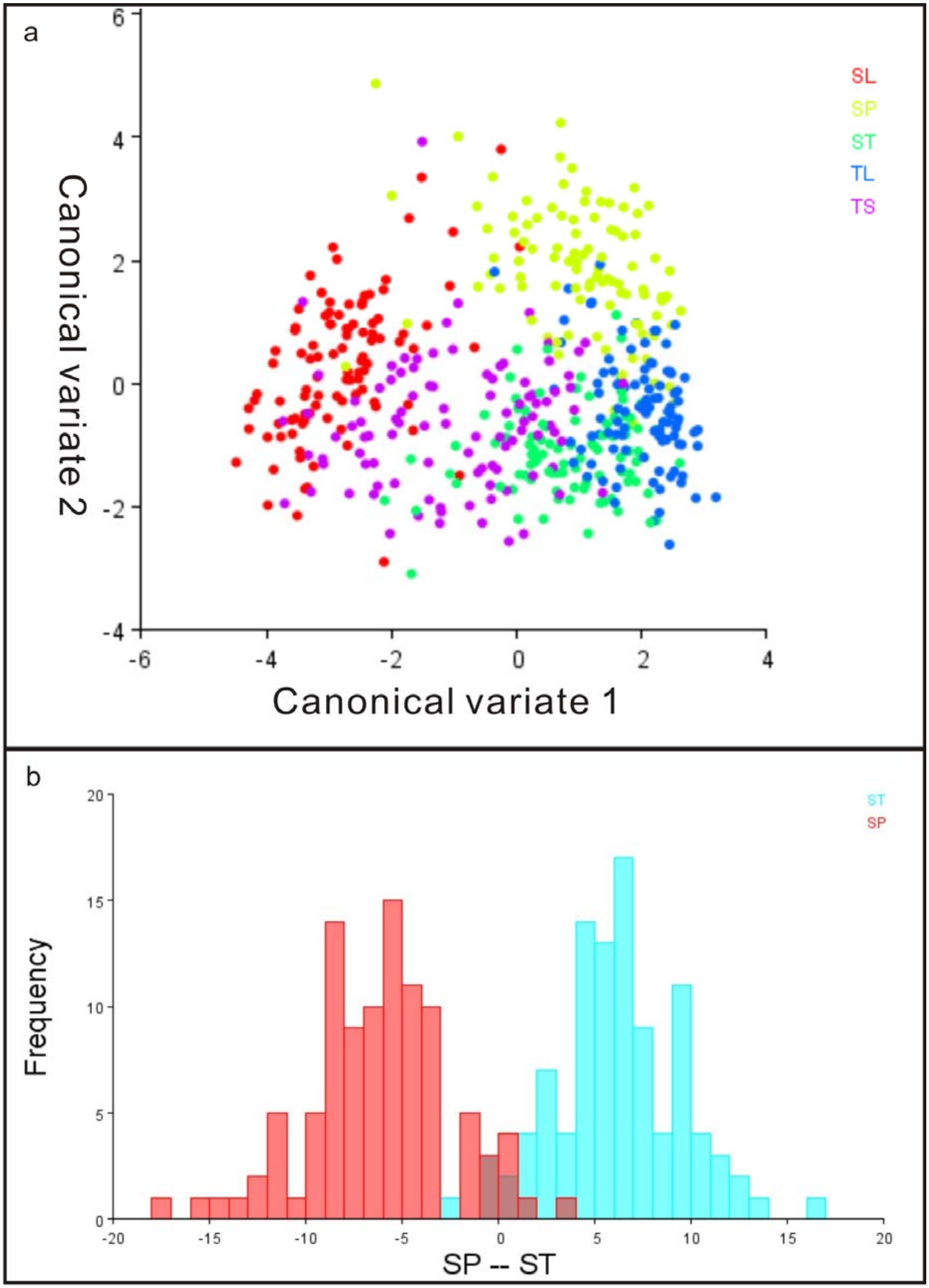

Fig. 5. (a) Scatterplot from CVA of the second data set consisting of the leaf shapes of *S. lichuanensis*, *S. potamogetifolia*, *S. trifolia* and its two varieties. (b) Discriminant analysis of the pair between *S. potamogetifolia* and S. *trifolia* using leaf shape. SL corresponds to *S. lichuanensis*, SP to *S. potamogetifolia*, ST to *S. trifolia*, TL to *S. trifolia* var. *longiloba*, and TS to *S. trifolia* var. *sinensis*.

Although *S. potamogetifolia* is endemic to China and only distributed in some wet lands of south China, it has many ecotypes with different types of leaves. The ratio of length to width of these leaves vary in a large range, which is similar as the condition in *S. trifolia*. Meanwhile we can find that there is a large overlap between the scatter of *S. potamogetifolia* and *S. trifolia* in the PCA diagram of first data set. As these similarities in leaf shapes, some authors even considered *S. potamogetifolia* as a hybrid between *S. trifolia* and another species (Rataj, 1972). But through our analysis of the second data set, these two species show the evident differences in the leaf shapes. With some large divergence between their reproductive structures, there can not be a direct genetic relationship between *S. potamogetifolia* and *S. trifolia*.

Conclusions

Our results indicate that there are interspecific differences between the leaf shapes of Chinese *Sagittaria*. As the types of leaves in *Sagittaria* are determined at the bud stage, these differences could mainly come from different developmental regulation (Wooten & LaMotte, 1978; Bloedel & Hirsch, 1979). However, the leaf shape of *Sagittaria* is partly responding to environmental conditions at least, and this morphological plasticity is frequently observed in some species (Wooten, 1986). With the help of the tools used in our study, precise relationship would be established between the variation of leaf shape and environment factors in the future. In addition, *Sagittaria* is distributed across the world (Bogin, 1955; Cook *et al*., 1974). However, our studies are restricted Chinese *Sagittaria* species, but the prospective studies are required to carry on the leaf shapes of *Sagittaria* worldwide, which will help us to improve our conclusion about *Sagittaria* morphology, and to understand the evolution of *Sagittaria* as well.

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