

# Leaf midrib outline as a diagnostic character for taxonomy in *Anthurium* section *Urospadix* subsection *Flavescentiviridia* (Araceae)

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**ABSTRACT** – (Leaf midrib outline as a diagnostic character for taxonomy in *Anthurium* section *Urospadix* subsection *Flavescentiviridia* (Araceae)). The external morphology of the midrib of the leaf lamina is used as a diagnostic character for the taxonomy of species of *Anthurium* subsection *Flavescentiviridia* (section *Urospadix*) (Araceae). Our aims in this study are: characterize using morphological analysis the variation of the outline of the midrib along the leaf lamina using 22 of the 35 species of the subsection; evaluate the potential of this variation as a diagnostic tool for taxonomy. For each species, the leaf lamina was divided into three pieces of identical length. The median point of each region was transversely sectioned and its outline drawn with the aid of a camera lucida and microscope. Five distinct types of outlines were classified using current terminology. Considering the difficulty of diagnosing many of the species, this character could be useful for the taxonomy of this subsection.

**Key words:** leaf, monocots, morphology, species diagnosis

**RESUMO** – (Morfologia da nervura central da lâmina foliar como caráter diagnóstico para taxonomia de *Anthurium* subseção *Flavescentiviridia* (Araceae)). A morfologia da nervura central da lâmina foliar é usada como caráter diagnóstico para separar espécies do gênero de *Anthurium* subseção *Flavescentiviridia* (seção *Urospadix*) (Araceae). Os objetivos deste estudo são: através de análise morfológica, caracterizar a variação da forma da nervura central ao longo da lâmina foliar usando 22 das 35 espécies da subseção; avaliar o uso potencial desta variação como ferramenta para diagnose taxonômica de espécies de *Anthurium* da subseção *Flavescentiviridia*. Para cada espécie, a lâmina foliar foi dividida em três regiões de igual comprimento. A nervura central no ponto médio de cada região foi cortada transversalmente e sua forma delineada com auxílio de câmara clara. Os diferentes tipos de nervura central foram classificados utilizando-se terminologia corrente. Considerando-se a complexidade taxonômica do gênero, este caráter pode ser útil para taxonomia desta subseção.

**Palavras-chave:** diagnose de espécies, folha, morfologia, monocotiledôneas

## Introduction

The Araceae comprises 2,823 species in 109 genera (Gonçalves 2005). *Anthurium*, described by Schott in 1829, is the largest genus in the family, with approximately 1,000 species (Coelho 2006). In 1878, Engler divided the genus into 18 sections, and in 1898 the same author defined six subsections for section *Urospadix*, one of which is *Flavescentiviridia*, that actually presents 34 of its 35 species occurring in southeastern Brazil (Coelho 2004).

Engler (1905) and Schott (1860) described and distinguished aroid species using the external morphology of the midrib at the central region of the leaf lamina but not indicating the leaf region (basal, median or distal part) assessed. Anatomical studies have revealed that this character can vary along the leaf lamina (e.g. Leo *et al.* 1997 in Rubiaceae).

The outline format has been evaluated for species diagnosis in plant families other than Araceae, commonly using petiole and scape cross sections (Ferraz & Costa 1985, Scatena & Giulietti 1999, Metcalfe & Chalk 1950, 1979, Hussin & Sani 1998, Godoy & Costa 2003). For the Araceae, some previous studies examined the possible application of the leaf midrib outline as a diagnostic tool for aroid taxonomy (Croat & Bunting 1979, Mayo 1986, Keating 2002).

*Anthurium* is, morphologically, one of the most diverse genera of Araceae at the species level. This makes it complex and difficult taxonomically (Coelho & Mayo 2007). To the best of our knowledge, there is no published study which evaluates the variation of the midrib outline along the leaf lamina as a diagnostic character for species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*. Our intentions here are:

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through morphological analysis, record the variation in outline along the leaf lamina of the midrib in 22 species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*; and evaluate the potential use of this variation for diagnostic purposes in these species.

**Material and methods**

Morphological analyses were performed using cultivated and herbarium specimens. Individuals from *Anthurium* section *Urospadix*, subsection *Flavescentiviridia* were collected in different locations in Brazil and cultivated under identical glasshouse conditions in the Rio de Janeiro Botanical Garden (RJBG), Brazil. Subsection *Flavescentiviridia* has 35 species, 22 of them cultivated in the RJBG (table 1). Only these cultivated materials were studied. The other species not studied here were *A. bellum* Schott, *A. bouдетii* Nadruz, *A. bocainense* Catharino & Nadruz,

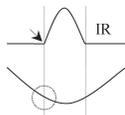
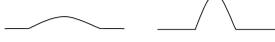
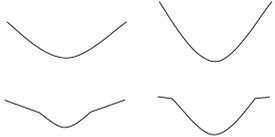
*A. fontellanus* Nadruz & Leoni, *A. fragae* Nadruz, *A. gomesianum* Nadruz, *A. jureianum* Catharino & Olaio, *A. langsdorffii* Schott, *A. miquelianum* K. Koch, *A. navicularis* Catharino & Nadruz, *A. pilonense* Reitz, *A. simonii* Nadruz, *A. unense* Nadruz & Catharino. Nomenclature followed Sakuragui & Mayo (1999) and Coelho (2004, 2006). All the studied species were analyzed using one sample obtained from fresh leaf collected in the field. Except for *A. regnellianum* Engl. and *A. victorii* Nadruz & Catharino which sample were obtained only from cultivated material, for 20 species (table 1), one or two other specimens were also analyzed using preserved leaves collected from herbarium specimens.

For each cultivated species, mature expanded leaves were collected. Fresh leaves were collected in the field, preserved in plastic bags, and brought to the laboratory. Preserved leaves from herbarium specimens were previously hydrated for 48hs before fixation (Kraus & Arduin 1997). Fresh and preserved leaves were fixed in FAA 70. For each species, leaf laminas were dissected from the petioles with a razor blade, measured with a rule and divided into proximal, median and distal pieces of identical length. For each piece, the mid-length at the midrib was determined. At this point, the entire midrib plus intercostal regions (0.5 cm width × 0.4 cm length each) was sectioned and separated into two pieces (0.5 cm width × 0.2 cm length). One piece was cross sectioned by hand with a razor blade, treated using standard techniques and stained with Safrablau (Johansen 1940). The other one was fixed in solution of glutaraldehyde 4% and formaldehyde 1% (McDowell 1978) in a sodium phosphate buffer 0.1M, pH 7.2, dehydrated in ethylic series, included in hydroxyethylmethacrylate (Gerrits & Smid 1983), sectioned at a 2-4 μm thickness using a Spencer microtome, and stained with Toluidine Blue O (O'Brien & McCully 1981). Photomicrographs were taken with an Olympus BX-50 light microscope and a Coolsnap digital camera. The outline of each section, including the entire midrib and both intercostal regions, was drawn at 40× magnification with the aid of a camera lucida.

Midrib outline was classified using current terminology described in Croat & Bunting (1979). These authors, proposing a standardization of *Anthurium* descriptions, classified midrib outlines as convex, concave, flat, V-shaped, U-shaped and omega-shaped.

In order to evaluate the potential application of the outline of leaf midribs in *Anthurium* for taxonomy a morphological key was constructed.

Table 1. Diagrams representing the outline types of the midribs found along the leaf lamina in 22 species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*. A general model is shown, indicating the junction of intercostal regions (IR) and midrib (M): angular not continuous (arrow) or not angular, continuous (circle). For each outline type, two examples representing possible variations along the same type of an outline are shown.

		
SURFACE	TYPE	DIAGRAMS
ADAXIAL	FLAT	
	U-SHAPED	
	CONVEX	
ABAXIAL	FLAT	
	CONCAVE	
	CONCAVE WITH KEEL	

## Results

For the majority of the species studied, the midrib outline varied along the leaf lamina (figures 1 - 23). This variation was different on the adaxial and abaxial surfaces. Only the types V-shaped and omega-shaped were not observed. However midribs with a keel were detected in some of the species studied. The outlines found were (tables 1, 2):

a) flat – midrib not prominent and at the same horizontal level or slightly down curved below of the intercostal regions. Very little upright folding. It occurs on both adaxial (figure 23) and abaxial surfaces (figure 28).

b) U-shaped – midrib plus both intercostals continuous but clearly folded upright. It occurs on the adaxial surface only (figure 24).

c) convex – midrib convexly prominent. Prominence varies from rounded to triangular (figures 25, 26, 28). Junction of both intercostal regions and midrib is usually angular, not continuous, but sometimes smoothly curved. It occurs only on the adaxial surface.

d) concave - midrib prominent. Prominence varies from U-shaped to triangular, from a slightly to a marked manner. It occurs only on the abaxial surface (figures 24, 25, 26).

e) concave with keel – identical to the concave type (midrib concavely prominent), but with cortical tissue projection toward the outside of midrib, constituting a keel (Figure 28). Junction of both intercostal regions and midrib is acute, not continuous. It occurs only on the abaxial surface.

The distribution of the types of midrib outline along the leaf lamina of the species examined is shown in tables 1 and 2. There were no differences in relation to the pattern of distribution of the midrib types found on cultivated and herbarium dry materials. Attention should be dedicated when analyzing the midrib outline at the distal region of *Anthurium* leaves. The outline type found at the mid-length at this region could be

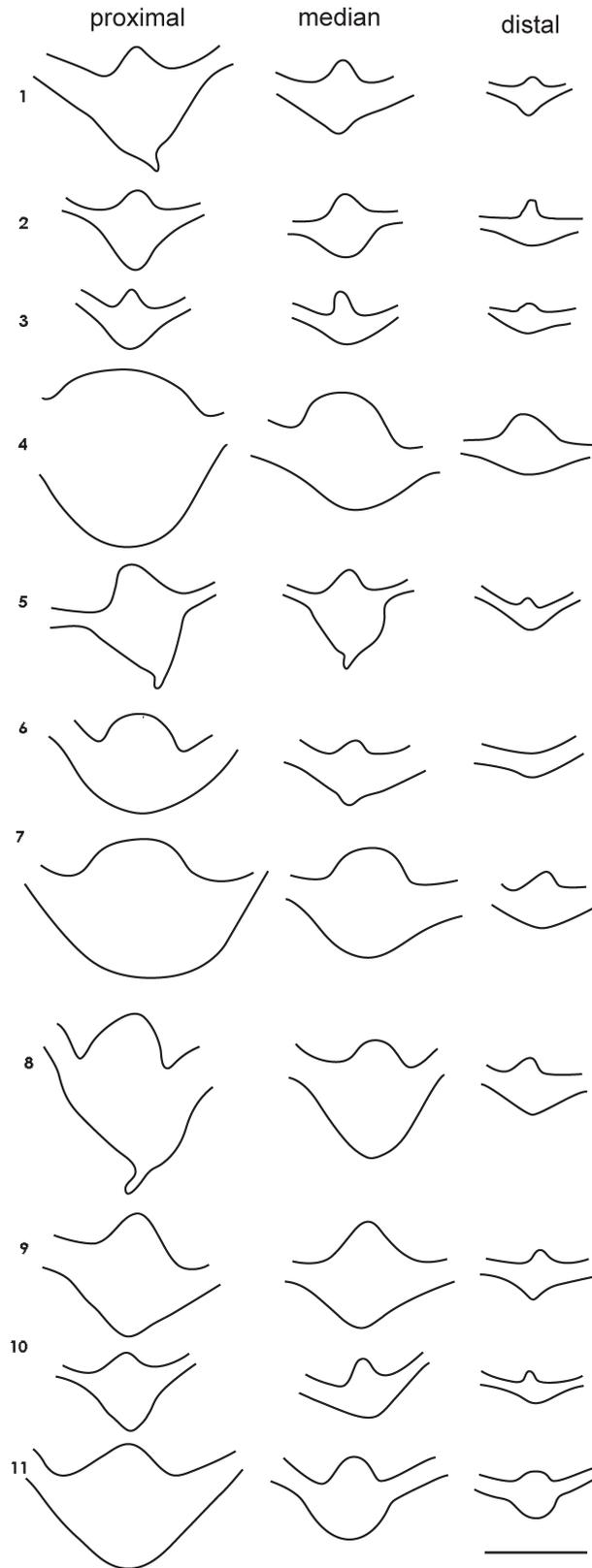
distinct from the one found more forward near to the leaf apex. For example, the adaxial surface of the midrib of *A. luschnathianum* is smoothly convex at the mid-length portion of the distal region (figure 15) but flat at the apex (figure 23).

On the majority of the concave midribs at abaxial surface, the junction of both intercostal regions and midrib was angular, not continuous (table 2). However, sometimes, this junction between intercostal region and midrib was continuous, constituting an U-shaped type at the abaxial surface, where midrib plus both intercostal regions were clearly folded upright. Considering that the distinction between both types (concave and U-shaped) could be arbitrary, both types were merged in the concave one.

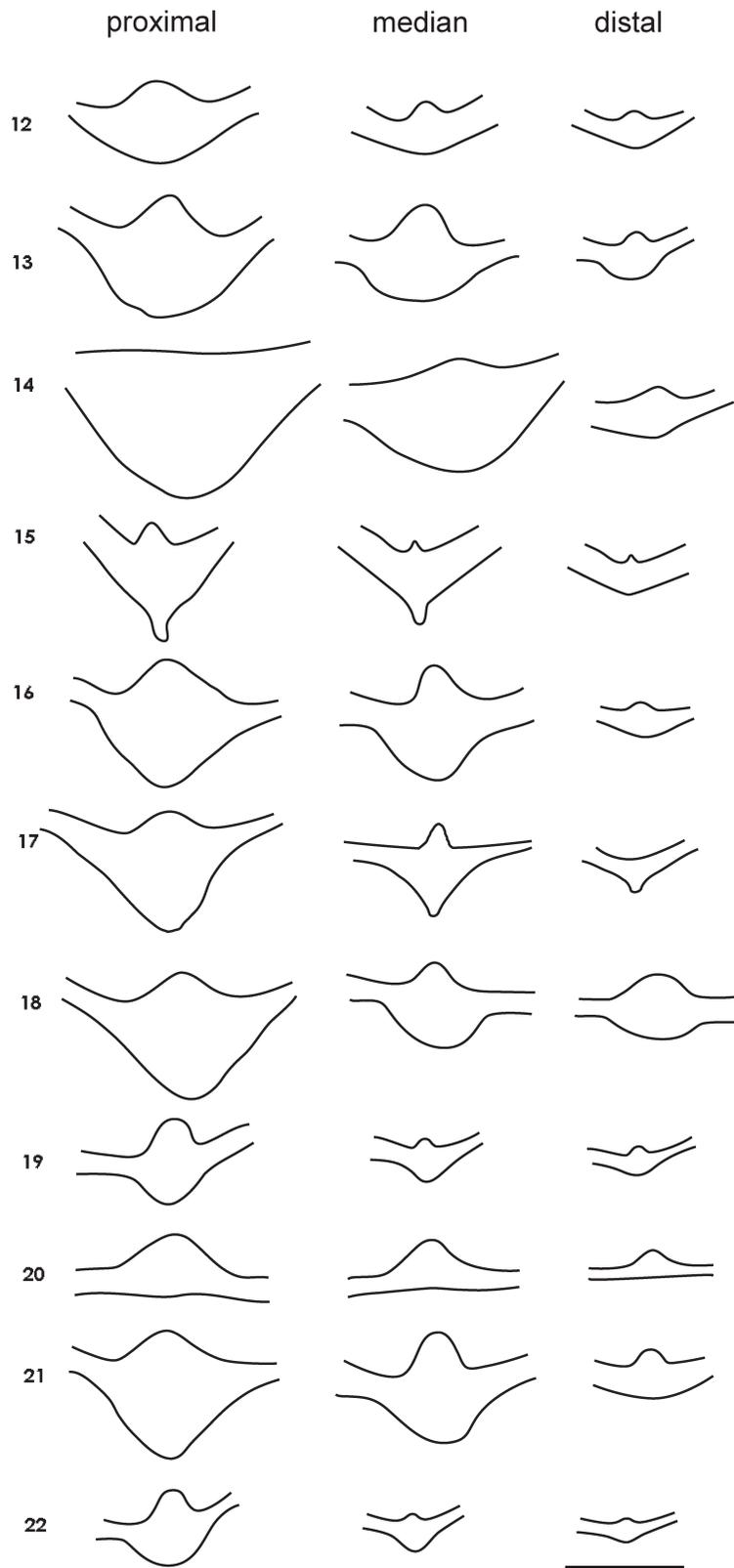
The potential application of the trait “variation of the midrib outline” for the diagnosis of *Anthurium* species was evaluated here. A taxonomic key was constructed and the 22 species studied could be separated into six distinct groups. Sixteen species presented the convex type at adaxial surface and the concave type at the abaxial surface along all the three portions of its leaves. Only five species presented some variation along the three leaf portions, and only at the adaxial surface. On *A. braggae*, *A. hoehnei* and *A. longicuspdatum* the adaxial surface varied from the convex type at the proximal region to the U-type at the distal region. Variation from the flat type at proximal region to the convex type at the distal region occurred on *A. luschnathianum*. Only *A. harrisii* and *A. mareense* presented the concave type with keel until the median portion of the midrib at the abaxial surface, while on *A. intermedium* and *A. urvilleanum* the concave with keel type was restricted to the proximal region of the midrib at the abaxial surface. The species *A. regnelianum* was the only one presenting a flat type of the midrib along the entire adaxial surface. This result show a potential application of this leaf character to characterize group of species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*.

Table 2. Matrix describing the variation of the midrib outline along the leaf lamina in 22 species of *Anthurium* section *Urospadix* subsection *Flavescensviridia*. Cultivated and herbarium materials presented the same results. Numbers indicate respective vouchers deposited in the herbarium of the Rio de Janeiro Botanical Garden (RB). Symbol (\*) indicates cultivated material used to obtain outline draws of the figures 1-22. Data are presented for surface (adaxial and abaxial) and region (proximal, median and distal). U = U-shaped; CV = concave; CK = concave with keel; CX = convex, F = flat.

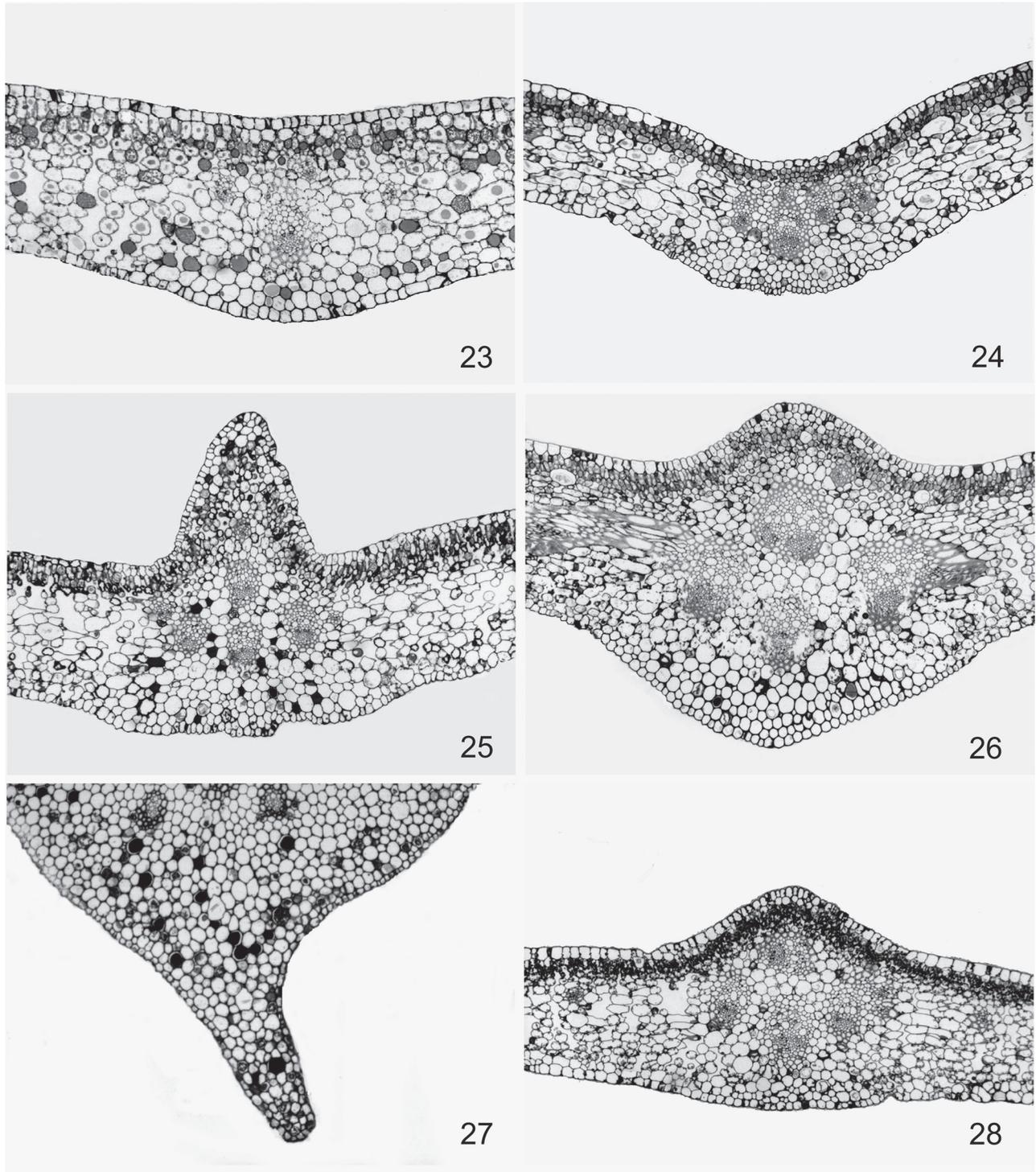
Species	RB	Proximal region						Median region						Distal region						
		Abaxial surface			Adaxial surface			Abaxial surface			Adaxial surface			Abaxial surface			Adaxial surface			
		CV	CK	F	U	CX	F	CV	CK	F	U	CX	F	CV	CK	F	U	CX	F	
<i>A. acutum</i>	378504*, 382564, 382564	X				X					X		X							X
<i>A. beyrichianum</i>	353489*, 429834		X			X					X		X							X
<i>A. brageae</i>	360300*, 354727	X				X					X		X							X
<i>A. comtum</i>	353492*, 378342, 378342	X				X					X		X							X
<i>A. harrisi</i>	353496*, 429522		X			X					X		X							X
<i>A. hoehnei</i>	378510*, 378496	X				X					X		X							X
<i>A. ianthinopodium</i>	371736*, 405464	X				X					X		X							X
<i>A. intermedium</i>	353666*, 432750		X			X					X		X							X
<i>A. jilekii</i>	368704*, 439475	X				X					X		X							X
<i>A. lucioi</i>	354724*, 422017	X				X					X		X							X
<i>A. loefgrenii</i>	365239*, 409454	X				X					X		X							X
<i>A. longicauspdatum</i>	378508*, 382566	X				X					X		X							X
<i>A. longifolium</i>	364757*, 432055	X				X					X		X							X
<i>A. luschnathianum</i>	376187*, 415323, 415325	X								X			X							X
<i>A. mareense</i>	378499*, 436153, 436153		X			X					X		X							X
<i>A. mourae</i>	364755*, 358574	X				X					X		X							X
<i>A. organense</i>	353650*, 359499, 451958	X				X					X		X							X
<i>A. parasiticum</i>	353495*, 42745, 422009	X				X					X		X							X
<i>A. parvum</i>	359314*, 469461, 451959	X				X					X		X							X
<i>A. regnellianum</i>	383406*				X	X					X		X		X					X
<i>A. sellowianum</i>	364273*, 430386, 429141	X				X					X		X							X
<i>A. victorii</i>	378507*	X				X					X		X							X



Figures 1-11. Midrib outlines from species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*. Each line represents one species and its respective midrib cross sectioned in the proximal, median and distal regions. 1. *A. acutum*. 2. *A. beyrichianum*. 3. *A. brageae*. 4. *A. comtum*. 5. *A. victorii*. 6. *A. harrisii*. 7. *A. hoehnei*. 8. *A. ianthinopodum*. 9. *A. intermedium*. 10. *A. jilekii*. 11. *A. lucioi*. Bar = 7600 $\mu$ m.



Figures 12-22. Midrib outlines from species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*. Each line represents one species and its respective midrib cross sectioned in the proximal, median and distal regions. 12. *A. loefgrenii*. 13. *A. longicuspidatum*. 14. *A. longifolium*. 15. *A. luschnathianum*. 16. *A. mareense*. 17. *A. mourae*. 18. *A. organense*. 19. *A. parasiticum*. 20. *A. parvum*. 21. *A. regnellianum*. 22. *A. sellowianum*.



Figures 23-28. Cross sections of leaf midribs of *Anthurium* species section *Urospadix* subsection *Flavescentiviridia*. 23. Adaxial surface flat, abaxial surface concave in the leaf apex of *A. luschnathianum*. 24. Adaxial surface U-shaped, abaxial surface concave in *A. longicuspidatum* (distal region). 25. Adaxial surface convex, abaxial surface concave in *A. beyrichianum* (distal region). 26. Adaxial surface convex, abaxial surface concave in *A. longifolium* (distal region). 27. Abaxial surface concave with keel in *A. harrisii* (median region). 28. Adaxial surface convex, abaxial surface flat in *A. regnellianum* (distal region). Bar = 350 $\mu$ m.

Taxonomic key based on the outline of the adaxial and abaxial midrib surfaces along the leaf lamina in 23 species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*

1. Abaxial surface proximal region flat ..... *A. regnellianum*
1. Abaxial surface proximal region concave or concave with keel
  2. Abaxial surface proximal region concave with keel
    3. Abaxial surface median region concave with keel ..... *A. harrisii*, *A. mareense*
    3. Abaxial surface median region concave ..... *A. urvilleanum*, *A. intermedium*
  2. Abaxial surface proximal region concave
    4. Adaxial surface proximal region flat ..... *A. luschnathianum*
    4. Adaxial surface proximal region convex
      5. Adaxial surface distal region concave ..... *A. bragae*, *A. hoehnei*, *A. longicuspidatum*
      5. Adaxial surface distal region convex ..... *A. acutum*, *A. comtum*, *A. ianthinopodum*, *A. jilekii*, *A. lucioi*, *A. loefgrenii*, *A. longifolium*, *A. mourae*, *A. organense*, *A. parasiticum*, *A. parvum*, *A. sellowianum*, *A. victorii*

### Discussion

Vegetative characteristics such as habit, phyllotaxy and leaf venation are important for description and identification of plant species. However the outline of plant organs in cross section is relatively less used for species diagnosis (Judd *et al.* 1999). Examples are the works of Nyffeler & Egli (2000) on the shoot and Wege (2001) on the scape. In relation to the leaf, both petiole and leaf lamina outline in cross section have already been applied to the diagnosis of plant species (Metcalf & Chalk 1950, 1979, Leo *et al.* 1997, Sajo *et al.* 1995).

However fewer references were found about the use of midrib outlines for species diagnosis (Mantovani & Pereira 2005). Bačić *et al.* (1992) compared the midribs of three species of *Arbutus* (Ericaceae) and Woltz *et al.* (1987) described the midrib outline prominence or concavity for 112 of the 184 species of Podocarpaceae. Both authors concluded that the midrib outline is a useful character for taxonomy.

In Araceae, Engler (1905), Schott (1893), Croat (1997), Keating (2002), and others have reported the usefulness of the midrib outline for the diagnosis of species. Keating (2002) described the midrib outline of leaf lamina in *Philodendron* (Araceae) that varies from ridged to concave on the adaxial surface but is less variable on the abaxial surface, varying from slight to highly convex. Our results in *Anthurium* detected five distinct types of midrib outline, but only three types occurred on the adaxial surface, and four on the abaxial surface. The same author stated that while highly variable, the midrib outline of the leaf lamina in *Philodendron* could be useful for the taxonomy of the genus. Although not testing this hypothesis for *Anthurium*, Keating (2002) reported the possible

application of the midrib outline for the diagnosis of the species in this genus.

This application was tested here for several *Anthurium* species from the section *Urospadix* subsection *Flavescentiviridia*. The variation of the midrib outline along the leaf lamina was useful for the diagnosis of the studied species, as exemplified by some taxonomical complexes in *Flavescentiviridia*. The species *Anthurium harrisii*, *A. intermedium*, *A. jilekii*, *A. sellowianum* and *A. urvilleanum* present similar morphological and reproductive characters that limit species diagnosis and separation. The shape of their leaves, widely used for the taxonomy of *Anthurium*, are highly overlapped. The same applies to the morphology of their inflorescences. But the differences in the outline of their midribs separate these five species into three distinct groups. In *A. hoehnei* and *A. mareense*, in addition to the plasticity of the leaf blade, secondary and tertiary ribs provide a bulate aspect for the leaf, making species delimitation confused, but midrib, in this case, confirmed the distinction of these species. Another example is represented by the species *A. luschnathianum* and *A. parasiticum*, which in the past were considered synonymous because of their very similar morphological and reproductive characters, nevertheless, the difference between the outline of their midribs reported here, reinforce the taxonomical distinction of both species.

Considering that *Anthurium* probably has a thousand species, some of them morphologically similar in the vegetative state, we suggest that the midrib outline in cross-section, along the leaf lamina, has high potential for the diagnosis and taxonomy of species of *Anthurium* section *Urospadix* subsection *Flavescentiviridia*.

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