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Morphoanatomical characters in the Nidularioid Complex (Bromeliaceae: Bromelioidae) from a phylogenetic perspective

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ABSTRACT

The Nidularioid complex, as formed by the genera *Nidularium* Lem., *Wittrockia* Lindm., *Neoregelia* L.B.Sm., *Canistropsis* (Mez) Leme and *Edmundoa* Leme, is a Bromelioideae group known for its difficult generic delimitation. This difficulty is attributed to the large number of nonexclusive characters that are used in these genera, which demonstrates their intimate relationship. In current phylogenies, the genera belonging to the Nidularioid complex always emerge as a unified group termed as 'Nidularioid Clade'. This study aims to reconstruct usual morphological characters in Bromelioideae, as well as anatomical characters from the leaf sheath and blade of species from the Nidularioid complex, in order to propose new synapomorphies for the group. To accomplish this, we proposed a phylogenetic hypothesis that used chloroplastidial *atp*B-*rbc*L, *mat*K, *trn*L-*trn*F and nuclear *PhyC* gene sequences obtained from the NCBI portal in a Bayesian analysis that resulted in a consensus tree. We also used parsimony and Bayesian methods to reconstruct previously delimited morphoanatomical characters. Our results indicate that the morphological characters typically used in the group's taxonomy represent homoplasies. Some anatomical characters are also homoplastic, such as the number of layers in the abaxial mechanical hypoderm from the leaf sheath. However, leaf anatomy provided new synapomorphies for the group, such as the presence of trichomes with elongated wing cells, the presence of adaxial epidermal cells with slightly thickened walls on the leaf blade and brachiform cells with long branches in the leaf sheath region. Thus, this paper presents new perspectives for future studies on the evolution of characters in the Nidularioid complex.

1. Introduction

The family Bromeliaceae belongs to the order Poales (APG IV, 2016), and its monophyletism is supported by morphological and molecular characters (Brown and Gilmartin, 1989; Benzing, 2000; Givnish et al., 2007, 2011). The family has a predominantly neotropical distribution, with the exception of *Pitcairnia feliciana*, which occurs in West Africa (Smith and Downs, 1974; Jacques-Fèlix, 2000). A recent study points out that the presence of *P. feliciana* on the continent of Africa probably results from long-distance dispersal by birds (Papini, 2017). Traditionally, Bromeliaceae is subdivided into three subfamilies, Pitcairnoideae, Tillandsoideae and Bromelioideae, distinguished from the one another by leaf margin, ovary position, fruit and seed types (Smith and Downs, 1974, 1977, 1979). With the advancements of molecular systematics, Pitcarnoideae was not shown to be monophyletic group, and Bromeliaceae was reorganized into eight subfamilies: Bromelioideae and Tillandsoideae that were maintained, Navioideae that was recircumscribed and Pitcairnoideae that was split into Pitcairnoideae *s.s.*, Puyoideae, Brocchinioideae, Hechtioideae and Lindmanioideae (Givnish et al., 2007; Givnish et al., 2011).

Among these subfamilies, Bromelioideae shows the greatest morphological diversity with its 33 genera, more than half of those belonging to the family, and ca. 936 species (Smith and Downs, 1979; Benzing, 2000;Butcher and Gouda, 2017). Species from this family are characterized by having aculeate leaf margin, inferior ovary, fruits as berries, and seeds without appendices (Smith and Downs, 1979). Its monophyletism is supported by morphological, cytogenetic and molecular characters (Givnish et al., 2007; Givnish et al., 2011). However,

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according to Smith and Downs (1974), genus delimitation is fragile, owing to substantial morphological diversity. The morphological traits commonly used to circumscribe the genera in this subfamily, such as the presence of petal appendices, branched inflorescence, and presence of pedicles, are homoplastic (Sousa et al., 2007; Schulte and Zizka, 2008; Aguirre-Santoro et al., 2016).

The Nidularioid complex is a Bromelioideae's group with particularly difficult delimitation (Leme, 1997). According to Leme (1997, 1998, 2000), the complex comprises the genera *Nidularium* Lem., *Wittrockia* Lindm., *Neoregelia* L.B.Sm, *Canistropsis* (Mez) Leme and *Edmundoa* Leme, and it is characterized by inflorescences with developed superior and primary scape bracts that accumulate water and resemble a nest over the foliar rosette. Nevertheless, the author highlights that the diagnostic characters for genera delimitation are both fragile and nonexclusive.

According to Wanderley et al. (2007), the challenges to delimitation of genera within the Nidularioid complex reflect the intimate relationship between them. When analyzing different species described in *Canistrum, Wittrockia* and *Edmundoa*, the authors also highlight that it is possible to notice a continuum of the characters considered as diagnostic, not supporting a separation of these genera. Although *Canistrum* is assigned to the Nidularioid complex based on its superior peduncle and primary involucral bracts, Leme (1997) further emphasizes that this genus is morphologically more similar to *Aechmea*. Among these similarities, the presence of asymmetrical sepals with membranous lateral wing and the presence of a pungent apex in its sepals are worth mentioning. This positioning can be verified in phylogenies of the subfamily Bromelioideae where species of *Canistrum* often emerge, along with species of *Aechmea*, in clades closely related to the Nidularioid clade (Silvestro et al., 2014; Heller et al., 2015).

Phylogenetic studies indicate that genera belonging to the Nidularioid complex are artificial (Silvestro et al., 2014; Evans et al., 2015). However, these genera always emerge as related to one another, with the exception of *Canistrum* (Silvestro et al., 2014; Evans et al., 2015; Heller et al., 2015), suggesting that they are a true taxonomic entity that is commonly referred to as “Nidularioid Clade” or “Nidularioid Complex”.

Morphological and anatomical studies have proved useful in the delimitation of taxonomic groups in Bromeliaceae (Hornung-Leoni and Sosa, 2008; Gomes-da-Silva et al., 2012), such as the presence of spiraled petals that are twined after anthesis and persistent in the apex of *Puya* fruits (Hornung-Leoni and Sosa, 2008), the presence of chlorophyllian parenchyma cells elongated anticlinally, the presence of a single-layered mechanical hypodermis in the abaxial portion in the *Vriesia corcovadensis* group (Gomes-da-Silva et al., 2012), the presence of mechanical hypodermis with intense wall thickening, and the presence of water-storage hypodermis in the abaxial and adaxial portions of the leaf in the clade enclosed by *Encholirium* + *Dyckia* (Santos-Silva et al., 2013).

In this sense, the present study examines possible morphoanatomical synapomorphies of the Nidularioid complex and its subclades, allowing new perspectives for studies of the phylogenetic relationships within this group.

2. Materials and methods

2.1. Taxon sampling

We sampled a total of fifteen species belonging to the genera that compose the Nidularioid complex *sensu* Leme (1997) for phylogenetic and morphoanatomical analyses (Table 1). *Acanthostachys strobilacea* (Schult. f.) Link, Klotzsch & Otto was included as an external group.

2.2. Sequence alignment and phylogenetic analyses

The chloroplast gene sequences of *atp*B-*rbc*L (797 pb), *mat*K (1747 pb), *trn*L-*trn*F (796 pb) and nuclear *Phy*C (1212 pb) were obtained from GenBank's NCBI portal, using the accession numbers provided by Silvestro et al. (2014), and they were aligned using Muscle software (Edgard, 2004). The sequences were manually checked using Mesquite 3.01 (Maddison and Maddison, 2015).

We used the Akaike Information Criterion (AIC) to select the best evolutionary model for each sequence, and GTR + G + I was selected. For the Bayesian analysis, we built a unique concatenated matrix, and each partition was named. The best evolutionary model (GTR + G + I) was applied to each partition of the matrix. Bayesian analysis was run in Mr. Bayes 3.2.6 (Ronquist et al., 2012) on the Cipres Gateway online server (http://www.phylo.org) (Miller et al., 2011). We ran the Markov Chain Monte Carlo (MCMC) chains for 10,000.000 generations, sampling every 1000th generation. The resulting phylogenies were summarized in a consensus tree after removing the first 500,000 generations as burnin (5%) (Appendix I).

Table 1
List of vouchers for taxa used for anatomical analyses.

Species	Voucher (Herbarium)
Aechmea disticantha Lem.	Leme 8.146 (HB), S.N.A.Miyamoto s/n (HUPG)
Aechmea turbinocalyx Mez	Leme 7.053 (HB)
Quesnelia arvensis (Vell.) Mez	Leme s/n (HB), F.M.C. Oliveira 39 (HUPG)
Quesnelia quesneliana (Brong.) L. B. Sm.	Leme s/n (HB), F.M.C. Oliveira 38 (HUPG)
Nidularium procerum Lindm.	Leme 990 (HB)
Wittrockia superba Lindm.	Leme 4.751(HB)
Neoregelia laevis (Mez) L.B.Sm.	Leme 4.752 (HB)
Neoregelia binotii (Mez) L.B.Sm.	Leme 3.482 (HB)
Neoregelia eleutheropetala (Ule) L.B.Sm.	Leme 1.976 (HB)
Edmundoa ambígua (Wand &Leme) Leme	Leme 756 (HB)
Edmundoa perplexa (L.B.Sm) Leme	Leme 2.956 (HB)
Canistropsis billbergioides (Schult. f.) Leme	Leme 4.918 (HB)
Canistropsis microps (E. Morren ex Mez) Leme	Leme 759 (HB)
Edmundoa lindenii (Regel) Leme	Leme 1.307 (HB)
Wittrockia cyathiformis (Vell.) Mez	Leme 6.411 (HB)

2.3. Morphological and anatomical data

We obtained morphological data from samples fixed in ethanol, as well as from the analysis of exsiccatae (herbaria SP, SPF, HUPG, MBM). For delimitation of anatomical characters, we used three individuals from each species from which we sampled three fully expanded leaves. The samples were fixed in FAA 50 (Johansen, 1940) and stored in ethanol 70°. We prepared anatomical slides using longitudinal and transversal sections of the medial third of the leaf sheath and blade, which were stained using Astra Blue and Fuchsin (Kraus and Arduin, 1997) and mounted in glycerinated agar (Kaiser, 1880 apud Kraus and Arduin, 1997).

We used Scanning Electron Microscopy (SEM) for leaf surface analysis. The samples were dehydrated in a gradual ethanol series up to absolute ethanol, submitted to critical CO2 point, and then submitted to vacuum gold metalization. After metallization, the samples were analyzed under a Shimadzu SSV-550 Scanning Electron Microscope (Silveira, 1989).

2.4. Ancestral state reconstruction

We used two methods for ancestral state reconstruction: parsimony in Mesquite (Maddison and Maddison, 2015) and Bayesian analysis using RASP (Yu et al., 2012). Both analyses were run using the consensus tree with the same topology as that proposed by Silvestro et al.