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BIOSSISTEMÁTICA DO COMPLEXO *Cryptanthus zonatus* (Vis.) Beer.

RAISSA ELIZABETH DE CASTRO MAGALHÃES

Dissertação de Mestrado
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Biossistemática do complexo C*ryptanthus zonatus* (Vis.) Beer.

Dissertação apresentada ao Programa de Pós-graduação em Sistemática e Evolução da Universidade Federal de Rio Grande do Norte, como parte dos requisitos necessários para a obtenção do título de Mestre em Sistemática e Evolução.

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Orientadora: Alice Calvente
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Aos meus pais:

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Resumo

A delimitação taxonômica ao nível de espécie em plantas não é uma tarefa fácil, devido ao grande polimorfismo dos vegetais. No presente trabalho objetivamos avaliar três morfotipos (formas) de *Cryptanthus zonatus* (Vis.) Beer (Bromeliaceae, Bromelioideae) descritos na literatura, lançando mão da biologia floral e fenologia, além da morfologia floral e anatomia foliar. As áreas de realização do estudo foram o Parque Estadual das Dunas de Natal e a Reserva Particular do Patrimônio Natural da Mata Estrela, no município de Baía Formosa Rio Grande do Norte (RN). Para o estudo fenológico foram feitas visitas mensais durante o período de um ano, onde foram feitas observações acerca das fenofases de floração e frutificação das populações das três formas de *C. zonatus*. Para biologia floral, procurou-se avaliar dados como: tipo de visitante floral, volume e concentração de néctar e horário de abertura e fechamento das flores. Flores das três formas foram coletadas em campo, analisadas ao estereomicroscópio e as medidas das peças foram feitas com a ajuda de um paquímetro. Cortes transversais e paradermais de folhas das três formas foram feitos, corados e posteriormente analisados ao microscópio ótico. Observações de cortes paradermais em microscópio eletrônico de varredura também foram feitas. Todos os dados mostraram não haver diferenças significativas entre as três formas. Desse modo, conclui-se que não há subsídios para o reconhecimento dos três morfotipos de *C. zonatus* como entidades taxonômicas, e que as ferramentas de fenologia, anatomia, biologia floral e morfologia floral não foram conclusivas para delimitar esses três morfotipos. Ainda visando caracterizar melhor a Flora de Bromeliaceae do RN, foi também estudada a anatomia foliar de *Orthophytum disjunctum*, uma espécie de um gênero irmão de *Cryptanthus*, apenas recentemente documentada no semiárido do RN. A comparação anatômica entre *Cryptanthus* e *Orthophytum* permitiu a separação dos gêneros com base na disposição dos estômatos e maior espessura do parênquima aquífero. Durante os trabalhos de campo, foi possível, ainda, documentar a primeira ocorrência de *Aechmea muricata* no RN, na RPPN Mata Estrela, auxiliando no entendimento da distribuição do táxon, que encontra-se ameaçado de extinção.

Palavras-Chave: Anatomia foliar. Fenologia. Morfologia Floral. Biologia Floral.

Abstract

The taxonomic delimitation at the species level in plants is not an easy task due to the large polymorphism of plants. In this project we aimed to evaluate three morphotypes (forms) of *Cryptanthus zonatus* (Vis.) Beer (Bromeliaceae, Bromelioideae) described in the literature using floral biology and phenology, as well as floral morphology and leaf anatomy. These studies were conducted in the Parque Estadual das Dunas de Natal, Rio Grande Norte (RN) and Private Reserve of Natural Patrimony Mata Estrela, in the municipality of Baía Formosa. The survey of the phenology of the morphotypes involved monthly specimen observation in the field, during one year. In each visit, we observed the status of flowering and fruiting phenophases of the population of the three forms of *C. zonatus*. For floral biology we sought to evaluate data like: observed floral visitors, nectar volume and concentration, time of anthesis and closing of flowers. Flowers of the three forms were collected in the field, analyzed by stereomicroscope, and measurements of the floral pieces were made with the help of a caliper. Transversal and paradermal sections of the leaves of the three forms were stained and then examined under an optical microscope. Observations of the epidermis under scanning electron microscopy were also conducted. The three morphotypes could not be sepated based on all evidence investigated. Thus, we conclude that there is not evidences to support the recognition of *C. zonatus* morphotypes as taxonomic entities, and also that the tools of phenology, anatomy, biology and floral morphology were not useful to delimit these three forms. Yet to characterize better the Flora of Bromeliaceae of RN, the leaf anatomy of *Orthophytum disjunctum* was also studied. *Orthophytum* is the sister genus to *Cryptanthus* and only recently documented in the semiarid of RN. The anatomical comparison between *Cryptanthus* and *Orthophytum* allowed the separation of both genera based on the arrangement of stomata and thickness of aquiferous parênquima. During the fieldwork, it was still possible to document the first occurrence of *Aechmea muricata* in RN, inside the Mata Estrela preserve, aiding the understanding of the distribution of the taxon that is currently threatened with extinction.

Keywords: Leaf anatomy. Phenology. Floral morphology. Floral biology.

SUMÁRIO

1. INTRODUÇÃO

1.1. A FAMÍLIA BROMELIACEAE

Bromeliaceae é uma das maiores famílias de monocotiledôneas de ocorrência neotropical, compreendendo 58 gêneros e aproximadamente 3.140 espécies (GIVINISH et al. 2011). Além da sua grande diversidade taxonômica, esse grupo merece destaque tanto pelo seu extraordinário papel ecológico (BENZING 2000, GINVINISH et al. 2004, 2007, 2011; HORRES et al. 2007), como pelo seu valor ornamental (HORRES et al. 2007). Estudos biogeográficos propõem que o ancestral de Bromeliaceae dissociou-se de seu grupo irmão Rapateaceae a aproximadamente 100 milhões de anos no escudo das Guianas, entretanto, seus eventos de divergência são mais recentes, datando de aproximadamente 19 milhões de anos. Posteriormente, há aproximadamente 10 milhões de anos, teria surgido o grupo de Bromelioideae (GIVINISH et al. 2011). O gênero *Cryptanthus*, que é alvo deste estudo, é mais recente, datando de aproximadamente 5,5 milhões de anos (GIVINISH et al. 2011). O surgimento desse grupo ocorreu durante o quaternário, quando mudanças climáticas (RULL 2006, 2011) e geológicas (ANTONELLI 2010) teriam direcionado eventos de diversificação biológica (RULL 2006, 2011), principalmente em Angiospermas (ANTONELLI 2009). No caso de Bromeliaceae esses eventos teriam ocorrido principalmente por irradiação adaptativa (CRAYN; WINTER; SMITH 2004). A irradiação adaptativa ocorre quando surgem oportunidades ecológicas através da dispersão para novos hábitats, e a aquisição de características chaves permite a rápida adaptação nesse novo ambiente (YODER et al. 2010; GLOR 2010). No caso de Bromeliaceae, a estrutura corporal em roseta, a presença de tricomas peltados absortivos, o metabolismo CAM e as folhas com características xeromórficas foram responsáveis pelo processo de diversificação do grupo em ambientes com restrição de água, e no ambiente epifítico (BENZING 2000, CRAYN; WINTER; SMITH 2004; SHULTE; HORRES; ZIZKA 2005, SHULTE; BARFUSS; ZIZKA 2009). Entretanto, reversões surgiram ao longo de sua história evolutiva (BENZING 2000, CRAYN; WINTER ;SMITH 2004; SHULTE; HORRES; ZIZKA 2005; SHULTE; BARFUSS; ZIZKA 2009), permitindo que táxons mais derivados ocupassem o sub-bosque e os troncos de árvores das florestas mais úmidas (BENZING 2000; CRAYN; WINTER ;SMITH 2004; SHULTE; HORRES; ZIZKA 2005; SHULTE; BARFUSS; ZIZKA 2009).

Tradicionalmente a família Bromeliaceae está dividida em três subfamílias: Pitcairnioideae; Bromelioideae e Tillandsoideae (BENZING 2000). Entretanto, análises moleculares revelaram que o antigo grupo Pitcairnioideae é parafilético, aumentando o número de subfamílias para oito (GIVINISH et al. 2004, 2007, 2011). Filogeneticamente, as subfamílias Bromelioideae e Tillandsioideae evoluíram mais recentemente, sendo assim, algumas das linhagens tradicionalmente tratadas como Pitcairnioideae, são hoje consideradas as mais antigas e consideradas como subfamílias independentes, com centro de diversificação no Planalto das Guianas (GIVINISH et al. 2007, 2011; SCHULTE et al. 2005). Apesar de sua grande riqueza de espécies e da literatura descrevê-la como monofilética com base em caracteres morfológicos (GILMARTIN; BROWN 1987) e moleculares (TERRY; BROWN; OLMSTEAD 1997; GIVINISH et al. 2004, 2007, 2011, SHULTE; HORRES; ZIZKA 2005, SHULTE; BARFUSS; ZIZKA 2009; HORRES et al. 2007), autores como (HORRES et al 2007) atentam para o fato da falta de revisões taxonômicas ao nível de gênero em Bromelioideae.

Entre as subfamílias, Bromelioideae é a que merece destaque por ser a mais complexa em termos taxonômicos e por ser a que possui o maior número de espécies. Ela inclui 32 gêneros e aproximadamente 800 espécies (LUTHER 2008), sendo um grupo marcante pela sua abundância na Floresta Atlântica brasileira (MARTINELLI 2008). Sendo assim, é comum encontrar casos onde a separação das espécies não é fácil, tendo o sistemata que recorrer a diferentes ferramentas. Tais grupos de espécies vêm sendo tratados como complexos. Diversas revisões vêm sendo propostas para táxons de Bromelioideae (e.g. CANELA; LOPEZ PAZ; WENDT 2003; SOUSA;WENDT 2008, FARIA; WENDT; BROWN 2010).

1.2. O GÊNERO *CRYPTANTHUS* OTTO & A. DIETR

Cryptanthus Otto & A. Dietr. compreende dois subgêneros (*Cryptanthus* e *Haplocryptanthus*) (RAMÍREZ-MORILLO 1996). O gênero é endêmico do Brasil, totalizando 77 espécies (FORZZA et al. 2013). Pertence à subfamília Bromelioideae, embora a sua situação filogenética seja alvo de controvérsias em razão de pesquisas que mostram que esse grupo tem um número cromossômico igual a 17 (n = 17), enquanto o restante da família possui n = 25 (RAMÍREZ-MORILLO 1996; COTIAS-DE-OLIVEIRA et al. 2000, RAMÍREZ-MORILLO; BROWN 2001). O gênero está intimamente associado à *Orthophytum* e *Lapanthus* (LOUZADA; VERSIEUX 2010) com base em dados moleculares (SHULTE; BARFUSS; ZIZKA 2009). Além disso, a presença de andromonoecia é destacada como um caráter diferencial em *Cryptanthus* (RAMÍREZ-MORILLO 1996; BENZING 2000). Dentro da família Bromeliaceae, esse gênero exibe características morfológicas e fisiológicas consideradas derivadas (RAMÍREZ-MORILLO 1996) e é notável por ocorrer em um grande conjunto de hábitats e por ser geralmente microendêmico, razão pela qual muitas de suas espécies estão ameaçadas (VERSIEUX; WENDT 2007).

1.3. ESPECIAÇÃO EM PLANTAS E A BIOSSISTEMÁTICA EM BROMELIACEAE

Antes de tudo é preciso ressaltar que o uso do conceito de espécie tem uma grande importância no que diz respeito à classificação, catalogação e a determinação das relações de parentesco da diversidade biológica (CRACAFT 1989; STACE 1989), entretanto, no caso de plantas, definir o que é espécie não tem sido fácil e encontrar um conceito unânime que sirva como parâmetro para definir essa entidade tem sido cada vez mais difícil (CRACAFT 1989; MACDADE 1995, RIESEBERG; WILLIS 2007; SOLTIS; SOLTIS 2009). No que diz respeito ao conceito morfológico, a dificuldade está no fato de a caracterização morfológica depender do taxonomista, sendo dessa forma extremamente subjetiva (CRACAFT 1989; STACE 1989; SOLTIS; SOLTIS 2009). O conceito biológico também é questionado porque grupos botânicos diferentes são frequentemente intercruzáveis, formando híbridos (RIESEBERG; WILLIS 2007; SOLTIS; SOLTIS 2009). No caso de híbridos, descendentes nem sempre são facilmente diferenciados morfologicamente de seus parentais (SOLTIS; SOLTIS 2009), ou expressam um fenótipo intermediário, aumentando ainda mais a dificuldade do uso do conceito morfológico (MACDADE 1995). A formação de híbridos pode ou não gerar adição de grupos cromossômicos (poliploidia ou homoploidia). Os poliplóides gerados por hibridização são chamados alopoliplóides (CRACAFT 1989; RIESEBERG; WILLIS 2007; SOLTIS; SOLTIS 2009**)**. Porém, a duplicação do número de conjuntos cromossômico também pode ser ocasionada via fecundação entre indivíduos de uma mesma espécie, formando autopoliplóides (CRACAFT 1989; SOLTIS; SOLTIS 2009). Dessa forma a poliploidia é destacada como sendo o principal processo evolutivo desencadeador da especiação em plantas, principalmente angiospermas (CRACAFT 1981; SOLTIS; SOLTIS 2009; SOLTIS et al. 2009).

É importante notar que a hibridização corre devido à quebra de barreiras reprodutivas (SOLTIS; SOLTIS 2009; RIESEBERG; WILLIS 2007), que por sua vez podem ser anteriores ou posteriores ao cruzamento (LOWRY et al. 2008). Segundo (LOWRY et al. 2008), as barreiras pré-zigóticas possuem uma maior importância no isolamento, por isso, estas serão destacadas nesse trabalho. Entre as barreiras reprodutivas pré-zigóticas, podemos citar fenologia, polinizador, geografia, competição por pólen, e sistema reprodutivo (LOWRY et al. 2008).

No cenário acima descrito merece destaque a biossistemática, também chamada de taxonomia experimental, a qual utiliza a análise de dados provenientes de diversos campos, tais como: genético, citológico, morfológico e ecológico, utilizados como auxiliares na tomada de decisões e na prática da taxonomia (STACE 1989). A biossistemática tem sido uma ferramenta vastamente utilizada em pesquisas taxonômicas em grupos botânicos (e.g. RIGGINS et al 1977, BERNARDELLO et al. 1995, CASIVA et al. 2002; CONCEIÇÃO et al. 2008), e é particularmente útil no estudo de grupos complexos e controversos, uma vez que leva em conta mais o plano populacional do que o específico, considerando as variações fenotípicas (STACE 1989). Na família Bromeliaceae, muitos estudos biossistemáticos têm sido desenvolvidos durante os últimos trinta anos (e.g. GILMARTIN; BROWN 1987; RAMÍREZ-MORILLO 1996; GINVINISH et al. 2004, 2007, 2011, SHULTE; HORRES; ZIZKA 2005, SHULTE; BARFUSS; ZIZKA 2009; HORRES et al. 2007), no intuito de compreender o relacionamento entre táxons problemáticos.

Por todos esses motivos explicitados acima, este trabalho recorre à abordagem biossistemática, utilizando caracteres morfológicos, anatômicos e ecológicos (fenologia e polinização) para avaliar com mais detalhes o complexo *C. zonatus,* sua classificação infraespecífica e relações com demais espécies do gênero, e assim discutir sua situação taxonômica e a viabilidade da utilização dos conceitos morfológicos e biológicos de espécie.

1.4. O COMPLEXO *CRYPTANTHUS ZONATUS* (VIS.) BEER

Tendo em vista que foi observado no Parque Estadual das Dunas a existência de três formas de uma espécie de *Cryptanthus*, e que a ocorrência desse gênero no RN ainda não era conhecida, a presente dissertação buscou reconhecer os limites entre os morfotipos (ou morfos de cor, ou *color morphs*) existentes na mata atlântica do RN e reconhecer se os táxons do RN seriam mesmo pertencentes a *C. zonatus*. Na literatura, observa-se que tais morfotipos são tratados como variedades (BAKER 1889) ou formas (SMITH; DOWNS 1979), como sinônimos (SIQUEIRA-FILHO; LEME 2006) ou apenas como morfotipos (RAMÍREZ-MORILLO 1996). Além da controvérsia quanto aos morfotipos, existe também uma complexa delimitação de espécies, envolvendo outros nomes de espécies tratadas como independentes, como *C. burle-marxii* e *C. fosterianus* (explicado no capítulo 1)

Cryptanthus zonatus é altamente ornamental e foi reconhecida a partir de coletas obtidas no estado de Pernambuco (BAKER 1889). A obra original, onde a espécie foi descrita em um gênero já sinomizado - *Pholidophyllum* -, não faz referência ao estado de Pernambuco (VISIANI 1847). Tampouco a obra de (BEER 1856), que foi quem apresentou a nova combinação, transferindo a espécie para *Cryptanthus*. Acreditava-se que sua distribuição geográfica se estendia de Alagoas a Pernambuco, no Nordeste do Brasil (RAMÍREZ-MORILLO 1996, SIQUEIRA FILHO; LEME 2006). No entanto, no presente trabalho, uma nova ocorrência de *C. zonatus* foi relatada para o estado do Rio Grande do Norte, ampliando sua área de distribuição, bem como para o gênero como um todo (VERSIEUX; MAGALHÃES; CALVENTE 2013).

- ***Cryptanthus zonatus* (Vis.) Beer, Die Familie der Bromeliaceen 76. 1856.**

Basiônimo: *Pholidophyllum zonatum* Vis. Ind. Sem. Hort. Patav. (1847) 4; et in Otto & Dietr. Allg. Gartenz. xvi. (1848) 30.

Neótipo: Caruaru/PE, Brazil. (*E. Waras s.n*, Data: 25 de Junho de 1972. HB), selecionado por (Ramírez-Morillo 1996).

A história taxonômica do que hoje chamamos de *C. zonatus* é um tanto confusa e agravada pelo fato da espécie ter sido descrita em um catálogo de sementes do horto de Pádua, Itália, por (VISIANI 1847), que apresentou uma descrição formal e detalhada do gênero (*Pholidophyllum*) e da espécie tipo (*P. zonatum*), mas não menciona a procedência ou um tipo preservado. A mesma descrição foi publicada por (OTTO; DIETRICH 1848). De acordo com (OTTO; DIETRICH 1848) o *P. zonatum* (basiônimo de *C. zonatus*) incluiria diversos nomes de plantas já em cultivo nos jardins botânicos da Europa, incluindo variedades e cultivares, sob o nome de *Tillandsia acaulis*. Os termos *zonata*, *zonatum*, ou *zebrina* que são citados por (OTTO; DIETRICH 1848) se referem a variedades ornamentais de *T. acaulis*, com a peculiar característica de apresentarem listras transversais ornando as folhas. (OTTO; DIETRICH 1848) citam que essas plantas eram listadas com diferentes nomes (quase todos nomes nus) pelos responsáveis pelos jardins botânicos, como, por exemplo: *Billbergia acaulis*, de follhas marrons ou folhas verdes; *Tillandsia acaulis zonata*; *T. zonata*; *T. acaulis zebrina* e *T. acaulis* verde, *T. zonata* folha branca e marrom; *T. zonata* (zebrina) folhas marrons e folhas verdes. Nessa descrição, também foram utilizados pela primeira vez os termos *fuscus* (que do Latim significa marrom, pardo) e *viridis* (verde), já que a espécie *P. zonatum* é apresentada pelo autor como tendo duas variedades: variedade *viridis* e variedade *fuscus*, ambas de uso comum nos jardins.

(BEER 1856) transferiu essas duas variedades para o gênero *Cryptanthus*, descrito primeiramente por (OTTO; DIETRICH 1836). Na sua descrição, Beer caracteriza a diferença entre essas duas variedades, nomeando-as como *Cryptanthus zonatus* var. *viridis* Beer e *C. zonatus* var. *fuscus* Vis. Este autor determina a diferença entre elas com a cor da folha. Ele também enfatiza que ambas possuem faixas transversais, e relaciona a semelhança da folhagem e da flor dessa espécie com as de *Cryptanthus undulatus* Otto; A. Dietr. *Cryptanthus undulatus*, no entanto, não é mais tida como uma espécie aceita, sendo atualmente tratada como sinônimo de *C. acaulis* e de *C. sinuosus* que é um táxon ainda impreciso quanto a sua existência taxonômica (SMITH; DOWNS 1979, RAMÍREZ-MORILLO 1996).

A primeira menção ao estado de Pernambuco foi apresentada por (BAKER 1889), ao indicar que a espécie fora introduzida por Quesnel ao Jardim de Paris em 1842, a partir de matrizes vindas do Brasil e posteriormente distribuida entre outros jardins europeus, inclusive Kew. (BAKER 1889) lista apenas a variedade típica e a var. *fuscus*, não fazendo menção à variedade *viridis*. Porém entende-se pela descrição que na circunscrição de Baker, a variedade típica teria folhas verdes.

(MEZ 1896) inclui duas formas (*viridi* e *fusca*) para *C. zonatus* e não aceita o nível variedade. Ele indica que a f. *viridi* encontra-se em cultivo no horto de Breslau (Polônia) e indica a procedência da espécie como Pernambuco, próximo à Berberibe. Em (MEZ 1934), *Cryptanthus* é tratado como tendo dois subgêneros, *Hoplocryptanthus* e *Eucryptanthus*, ao qual *C. zonatus* pertence. A espécie é descrita da mesma maneira que em (MEZ 1896), com duas formas, *viridis* e *fusca*, porém o autor chama atenção à mudança da coloração na forma *fusca*, a qual, segundo ele, é palidamente avermelhada/acastanhada durante o inverno e vermelha durante o verão. A cor da folha é a única coisa que a difere da forma que possui folha verde e destaca que ambas estão em cultivo. Tais informações são importantíssimas, pois se trata do relato mais antigo de que as cores das folhas mudam em cultivo, de acordo com a temperatura.

(SMITH 1956) usa pela primeira vez a forma *zonatus* e apresenta uma chave de identificação das formas. As plantas de folhas verdes ou alvacentas, devido aos tricomas, são divididas em duas formas: f. *zonatus*, com a superfície abaxial densamente recoberta de tricomas e f. *viridis*, com a face abaxial verde, glabra. A forma *fuscus* é dada aos espécimes de folhas tingidas de vermelho, mas sem mencionar estrias ou zonas de tricomas. Em (SMITH; DOWNS 1979), o complexo é tratado taxonomicamente da mesma forma que na obra de (SMITH 1956).

(RAMÍREZ-MORILLO 1996) descreve o complexo e menciona a existência de três formas, porém, conclui que, devido à escassez de material desse complexo, não há indicativo da existência das três formas na natureza, só em cultivo. Por isso, trata as três formas como sinônimos, e sugere que mais estudos de campo devem ser realizados para confirmar a existência real dessas três formas na natureza e, dessa forma, validar taxonomicamente os nomes subespecíficos. A autora propõe que uma nova seção, a ser chamada Sect. Zonatae fosse descrita para incluir as espécies de folhas acastanhas e com margens onduladas e tricomas formando estrias transversais argêntesas. Tal seção incluiria *C. burle-marxii, C. fosterianus* e *C. zonatus*.

Em (SIQUEIRA-FILHO; LEME 2006), a espécie é caracterizada como em (RAMÍREZ-MORILLO 1996), sendo distinguida das demais por apresentar faixas transversais. Os autores fazem menção a variação da cor das folhas, que podem ser verdes ou marrons, e que os indivíduos com essas variações crescem juntos, entretanto não relacionam as variações morfológicas com categorias infraespecíficas, como forma ou variedade. Também fazem menção a uma variação da espécie, com folhas verdes e a superfície adaxial quase totalmente glabra, possuindo apenas alguns resquícios das faixas transversais de tricomas na parte basal da folha, porém também não nomeiam essa variação. Os autores apresentam informações sobre a biologia floral e fenologia do táxon.

Além da controvérsia em relação à variação morfológica e classificação infraespecífica de *C. zonatus*, também é destacada na literatura a íntima semelhança morfológica dessa espécie com outras espécies do gênero, como é o caso de *C. dianae, C. burle-maxii* e *C. fosterianus*, indicando que estudos adicionais são necessários para estabelecer os limites infraespecíficos e específicos de *C. zonatus* (SMITH 1952; RAMIREZ- MORILLO 1996, SIQUEIRA-FILHO; LEME 2006).

2. ORGANIZAÇÃO DA DISSERTAÇÃO

Esta dissertação está organizada em capítulos. Buscou-se aqui caracterizar as populações de *C. zonatus* do RN, utilizando uma abordagem biossistemática.

No **capítulo 1** é apresentado o primeiro registro do gênero para o RN e observações de variação fenotípica nas populações do Parque Estadual das Dunas e na RPPN Mata Estrela. No **capítulo 2** é apresentada a anatomia foliar dos morfotipos ocorrentes no RN. Os resultados indicam não haver variação da organização interna dos tecidos e subsidiam que todos os três morfos de cor sejam tratados como uma só entidade. No **capítulo 3** são apresentados os resultados da morfologia e biologia floral, que indicaram semelhança entre as características da espécie e seus morfotipos. No **capítulo 4** (**apêndice**), apresenta-se a primeira ocorrência de *Aechmea muricata* no RN, sendo esse um resultado secundário da dissertação, porém que visa ampliar o conhecimento da família no estado.

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3. OBJETIVOS

3.1. OBJETIVOS GERAIS

- Analisar a delimitação taxonômica do complexo *Cryptanthus zonatus* usando morfologia, biologia floral e anatomia.

3.2. ESPECÍFICOS

- Avaliar a distribuição geográfica do complexo *C. zonatus* Beer
- Avaliar o padrão morfológico foliar *Cryptanthus zonatus* e *Orthophytum disjunctum*.
- Avaliar o padrão morfológico floral do complexo *Cryptanthus zonatus*
- Caracterizar o padrão fenológico do complexo *Cryptanthus zonatus.*
- Estudar a biologia floral do complexo *Cryptanthus zonatus*.
- Comparar a síndrome de polinização e os polinizadores do complexo *Cryptanthus zonatus*.
- Incrementar o conhecimento da Flora de Bromeliaceae no estado do Rio Grande do Norte principalmente no que diz respeito a novas ocorrências, obtidas no trabalho de campo.

4. Chapter 1: Extension of the *Cryptanthus* range in Northeastern Brazil with new findings in the phenotypic variation including changes in the trichome's distribution, thus enhancing the understanding of the *Cryptanthus zonatus* complex (Bromeliaceae)

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Abstract

In this paper we describe the first occurrences of the genus *Cryptanthus* in the state of Rio Grande do Norte (RN), establishing a new northern distribution limit for the genus. Additionally, we report the finding of three forms of *C. zonatus* growing in sympatry within two different localities in Rio Grande do Norte along with the phenotypic variation in this species complex including changes in trichome's distribution. *Cryptanthus zonatus* is one of the most popular bromeliads due to its banded leaves. Nevertheless, the fact that it exists in two distinct glabrous-leaved forms is not readily accepted by all the authors. Confusion could be attributed to the fact that they were described from cultivated material without any information about provenance. Based on extensive fieldwork and observations we document here that banded leaves can become glabrescent and that green and red-maroon leaves (treated as forms) may occur on the same shoot. Our finding is important for future biogeographic works when considering Bromelioideae genera distribution in Eastern Brazil center of diversification. This leads to a better understand of species delimitation within this poorly known complex and would impact conservation strategies, given that all the species related to *C. zonatus* are under threat.

Key words: Atlantic forest. Bromelioideae. Color morphs. Endemism. Earth stars. Restinga.

4.1. INTRODUCTION

Bromeliaceae is a key element in the Neotropical landscapes, however species definition in several groups may be difficult and taxa complexes are constantly being revised (e.g., FARIA; WENDT; BROWN 2010; VERSIEUX 2011). The existence of distinct color phenotypes for one species in a single population (called color morphs), is documented under natural conditions for some species (e.g., BARBARÁ et al. 2007). On the other hand, ornamentation due to discolourous pigmentation in leaves of a single specimen is a frequent characteristic within several genera (e.g., *Neoregelia, Vriesea*), and this has defined new cultivars much appreciated by horticulturists (BENZING 2000a). Leaf color varying from green to deep wine red in a single species of bromeliad is also traditionally accepted as a consequence of growing conditions, particularly sun light (RAUH 1990). Basically, (for other than yellow, green and white) this is a consequence of anthocyanin accumulation in leaves, which may be related to abiotic or biotic effects such as extensive light, UV-B radiation, nutrient deficiency, reactive oxygen molecules, water stress (osmoregulation), and herbivory (CLOSE; BEADLE 2003; MANETAS 2006), which may be avoided due to aposematic coloration in red leaves (COONEY et al. 2012). In the genus *Alcantarea,* juvenile rosettes already show the color segregation pattern and color morphs (green and red individuals) may represent separate genetic clusters (BARBARÁ et al. 2007), suggesting the necessity to keep studying these kind of plant variations in order to understand their genetic base. In the present paper we document and discuss the color morphs existent in *Cryptanthus zonatus* (VISIANI 1847; BEER 1856). This may have confused taxonomists in the past who described distinct phenotypes as new taxa. We further present the first vouchered records of all the color morphs of *Cryptanthus zonatus* in Rio Grande do Norte state, which now is the most northern recorded distribution limit of this genus.

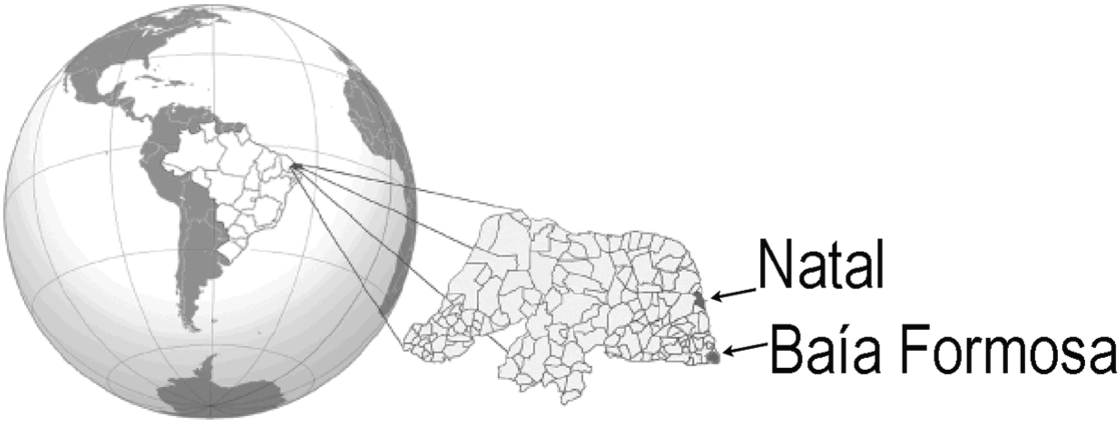
Cryptanthus (OTTO; DIETRICH 1836) comprises around 70 species and is a genus endemic to Brazil (SMITH; DOWNS 1979; LUTHER 2010; VERSIEUX et al. 2010; GOUDA; BUTCHER; GOUDA 2012). According to the recently published Brazilian list of plants, *Cryptanthus* can be found in the Northeastern region of this country, along the states of Paraíba, Pernambuco, Bahia, Alagoas, and Sergipe and in the Southeastern region, where most species occur, along Minas Gerais, Espírito Santo, and Rio de Janeiro states (FORZZA et al. 2013). *Cryptanthus* species are worldwide cultivated, popularly known as “earth stars” and congregate collectors into specialist horticultural societies, such as the *Cryptanthus* Society (STEENS 2007). Species of *Cryptanthus* are reported as being pollinated mainly by insects, particularly Euglossinae bees (BENZING 2000a, SIQUEIRA-FILHO; LEME 2006). Besides the sexual reproduction, asexual or vegetative reproduction occurs by axillary stolons (BENZING 2000a). *Cryptanthus* differ from most bromeliads due to the production of usually white and inodorous flowers and is among the few Bromeliaceae genera possessing dioecy. *Cryptanthus* can grow under the canopies of evergreen forest, tolerating low light and growing within the leaf-litter substrate (BENZING 2000b). Discontinuous trichome cover can produce striking displays as horizontal banded leaves as may be seen in *Cryptanthus zonatus*.

Less than the 20000 specimens of vascular plants representing the Flora of Rio Grande do Norte state are held within its two major herbaria collections, namely MOSS and UFRN (Thiers, [continuously updated]). Within this state, the historical lack of investment in extensive and intensive research programs focused on biodiversity inventories and limitation in the number of graduate programs devoted to form locally trained botanists are two of the main causes of this scenario. As a consequence, Rio Grande do Norte is listed as one of the poorest Brazilian states in terms of species numbers, harboring 1259 species. This is clearly interpreted as a consequence of poor botanical sampling and lack of investigations rather than actual species poverty (FORZZA et al. 2010). Besides the *caatinga* (the dry woodland from northeastern Brazil) that covers most of Rio Grande do Norte area, this state is also a landmark as the northern limit of the coastal Atlantic forest, which is considered to be a hotspot full of endemic bromeliads and the center of diversification of *Cryptanthus* (MARTINELLI et al. 2008). In this paper we describe the first occurrence of *Cryptanthus* in Rio Grande do Norte state, a finding that we consider important in evaluating the biogeography of the genus, the connectivity of the coastal Atlantic forest, as well as to add material to our scientific collections allowing more understanding of this genus given the paucity of specimens in herbaria (cf. MARTINELLI et al. 2008). Additionally, we indicate the *Cryptanthus zonatus* complex needing to be further revised. We expect that this data will allow the establishment of a better conservation status for the taxon and will further characterize species of the herbaceous stratum found amongst the vegetation of sand dunes in Rio Grande do Norte. These plants have received insufficient attention in previous floristic works (QUEIROZ; LOIOLA 2010).

4.2. MATERIAL AND METHODS

Since the year 2010 collections have been made along the Parque Estadual das Dunas de Natal (PEDD), a reserve of 1172 hectares located inside the city of Natal, capital of the state covered by Atlantic Forest and the coastal sand dunes shrubby vegetation (Fig. 1). Each population of *Cryptanthus zonatus* was georeferenced using a GPS and photographs were taken in the field to document phenotypic variability. Individuals have been collected and dried specimens were deposited in the herbarium UFRN (UFRN 14066, 14169, 14840, 14841). Additionally, field expeditions to the Private Reserve Mata Estrela (PRME), located at Baía Formosa, close to the border of Rio Grande do Norte and Paraíba states (Fig. 1) were carried out and specimens collected there (*Magalhães 18, 20* UFRN) are now cultivated in the garden of the Department of Botany, Ecology and Zoology of the Federal University of Rio Grande do Norte. The herbaria RB and UFRN were also inventoried for *Cryptanthus*. Floral analysis, particularly the measuring of bracts and sepals was made in individuals collected and fixed in ethanol 70% (N = 5). Notes on the phenology were taken during field work.

Figura 1 - Map of Rio Grande do Norte state indicating the two new occurrences of *Cryptanthus zonatus*.



Source: DIVA

4.3. RESULTS

So far seven populations of *C. zonatus* have been located within the PEDD, where at least three different color morphs can be seen in sympatry (Fig. 2 B–F). Plants may present the adaxial surface of the leaves totally green (forma *viridis*) to dark wine-red almost maroon (forma *fuscus*) or, typically, it can show white bands of trichomes, explaining the *zonatus* epithet (i.e., divided into zones). We documented variability in blade width, particularly for the green color morph, with plants having linear or almost obovate blades. The same was observed for the adaxial indumentum, which may fall with the aging of the leave that becomes nearly glabrous (Fig. 2 B–C). In PEDD, plants were found growing on sandy soil, at 50–80 m elev., usually along shaded areas. In PRME in the southern limit of Rio Grande do Norte (RN) (70 km south from Natal) we found larger populations then those in PDD (Fig. 2 A). At this place one specimen was collected (*Martinelli 15081*, RB herbarium) a few years ago but remained undetermined. There we also found the three distinct color morphs (Fig. 2). The measuring of bracts and sepals carried out for the flowers available here (N = 5) resulted in the bracts mean length = 1.87 cm, and sepals mean length = 2.8 cm. These results obtained indicate that our specimens would fit within the concept of *C. zonatus*. Our preliminary results on the phenology and flower biology of *C. zonatus* complex in PDD indicate that the red color morph blooms in March, April and October, and that anthesis occurs between 5:30–6:00 a.m. The green color morph was collected with fruits in August. The *zonatus* form individuals so far observed were blooming in March.

4.4. DISCUSSION

Cryptanthus zonatus was described from plants collected in the state of Pernambuco, where the species appears to be locally rare and is considered important as an ornamental (MENDES; GOMES; ALVES 2010; SIQUEIRA-FILHO; MACHADO 2001; SOUSA; WANDERLEY 2000). After locating blooming individuals in Rio Grande do Norte we considered it important to further investigate this first occurrence, given the lack of knowledge relating to populations of this taxon regardless of its ornamental / conservational importance.

The new occurrence is reported here for an urban reserve, PEDD, as well as for the PRME assuring that populations are protected. Previous floristic work indicates that at least 350 plant species occur within the PEDD (FREIRE 1990), however no reference has been made to *Cryptanthus*. This could be a consequence of low sampling, since populations are fragmented and sometimes shoots are difficult to be seen among the leaf-litter (Fig. 2). Our preliminary phenological observations also indicate that few individuals bloom at a time and flowers are short-lived. As documented for other southern areas (e.g., Pernambuco and Alagoas states, Siqueira-Filho & Leme 2006) we found three color morphs growing in sympatry or very close to each other: silver banded maroon leaves, green leaves, and glabrous red/maroon leaves, intermediary forms are also seen between these three patterns. We also observed that older leaves of the banded form may become glabrous.

FIGURE 2. A. Two color morphs (maroon and green – indicated by the arrow) growing intermingled in the understory of Mata Estrela. B. Maroon color morph plant growing next to a banded individual that present a basal glabrous leaf (arrow) at Parque das Dunas. C. Signs of trichome loss on the older leaves (arrow), by a maroon banded individual from Parque das Dunas. D. Green individual showing dark red/maroon marks on the base of the leaves, Parque das Dunas. E. Two rosettes showing similar pigmentation (green along the center and dark red/maroon at the margins) but contrasting in the banded-lepidote versus glabrous adaxial surface. F. Individual displaying the colors green and wine-red spots (scale = 14 cm). G. Blooming individual of the banded form presenting a staminate flower. H. Bisexual flower of the maroon morph. I. Basal stolon (arrow), in the maroon morph. J. Basal stolon (arrow), in the banded morph.

Source: Digital camera, Own made

This occurrence highlight the lost connectivity of the coastal forest of northeastern Brazil, which are nowadays completely fragmented and also stresses the need for PEDD and PRME to protect patches of the Atlantic rainforest, since this biome is a hotspot and has been progressively reduced and is now severely fragmented (RIBEIRO et al. 2009). New species, and even a recently described new genus (CABRAL et al. 2012) of other biological groups have been reported for PEDD indicating the necessity for more research within this reserve.

Due to difficulties with identification and to assure the plants from RN were, in fact, *C. zonatus* and not another species we analyzed the descriptions from (SMITH 1952 and SMITH; DOWNS 1979; RAMÍREZ-MORILLO 1996; SIQUEIRA-FILHO; LEME 2006) and the results are the following: (SMITH; DOWNS 1979) in their identification key highlighted the differences between *C. zonatus* vs. *C. fosterianus* (SMITH 1952) by the size of the sepals (19 mm vs 8 mm respectively), the free sepal lobes shapes and margins (acuminate, auriculate, entire vs. acute and apiculate, broadest at base, serrulate) and by the leaf texture (relatively thin and flexible vs. thick, fleshy and rigid). These authors also recognize three forms, namely *C. zonatus* f. *zonatus, C. zonatus* f. *viridis* (MEZ 1934), and *C. zonatus* f. *fuscus* (VISIANI 1847, MEZ 1896), the two later were described from cultivated material with unknown distribution in Brazil and were recently synonymized under *C. zonatus* by (SIQUEIRA-FILHO; LEME 2006).

(RAMÍREZ-MORILLO 1996) who firstly recognized this species complex indicated that a section should be created to place species having a unique pattern of transversal bands of trichomes on the blades. This section/complex would group, besides *C. zonatus* and *C. fosterianus, C. burle-marxii* LEME (1990: 12). Ramírez-Morillo further highlights that some characters previously used, such as the production of stolons is not useful to segregate taxa. Here we also observed that the same color morph may produce stolons or not. On the other hand, she considered the leaf texture, color of the blades, and the relative lengths of the sepals/floral bracts as taxonomically important. Ramírez-Morillo also stresses that further field work would be extremely valuable in order to understand if there are three different taxa or a single very variable one. In her identification key, taxa were separated by blade width, serration of the floral bracts, sepal length, floral bract/sepal length ratio (bracts about equaling the sepals or half as long as the sepals).

According to (SIQUEIRA-FILHO; LEME 2006), the differences between *C. zonatus* and *C. fosterianus* are the size of floral bracts, that are longer in *C. zonatus*, the margins of the sepals that are serrate in *C. fosterianus* and minutely denticulate or smooth in *C. zonatus*, and texture of the leaves, that are more leathery and fleshy in *C. fosterianus* than in *C. zonatus.*

Although the size of sepals analyzed, is a little bit longer than given in the descriptions and the floral bract appears to be minutely serrulate at the apex, the ratio of sepal/bracts length and the sepal apex, that are the characters highlighted by (SMITH; DOWNS 1979) and (SIQUEIRA-FILHO; LEME 2006), fit specimens from RN within the *C. zonatus* concept. Using the identification key provided by (CÂNDIDO 1995) who also mentioned that the genus would occur in RN, we could not key out to *C. zonatus*, since this author stress that *C. zonatus* does not have stolons. The segregation of *C. burle-marxii* from *C. zonatus* also has been based upon the presence of long and slender stolons versus short axillary shoots (SIQUEIRA-FILHO; LEME 2006). In the field we observed that this is a variable character, of low utility to separate specimens even in the same population (Fig. 2 I–J), which may or may not produce such stem in the same habitat.

Cryptanthus zonatus was recorded in Pernambuco state also in different habitats, these being *caatinga* where the designated neotype collection came from (RAMÍREZ-MORILLO 1996) as well as from patches of Atlantic forest isolated on the top of mountain ranges known as *brejos* (SIQUEIRA-FILHO; LEME 2006). In these fragments of Atlantic forest, the species grows in clay soil, which is different to RN populations that grow in litter-rich sandy soil. Currently, *C. zonatus, C. fosterianus,* and *C. burle-marxii* are in the the list of Endangered Species of the Brazilian flora. *Cryptanthus fosterianus* is listed as Critically Endangered, while *C. burle-marxii* and *C. zonatus* are listed as Vulnerable (CNCFlora, 2013).

4.5. CONCLUSION

Rio Grande do Norte state has a new occurrence for *C. zonatus* extending the range of this genus toward north. Here we added more data on the discussion about this poorly known species, particularly photos from populations in the field presenting different pigmentation or indumentum. Our work documented that this taxon is polymorphic and that in RN its populations are protected within two reserves with large populations. Single individual may have green or maroon leaves, and the typical banded form may lose trichomes with aging. Such phenotypic variability should be considered in order to avoid taxonomic confusion. However, field studies are still necessary to document the reproductive strategies of the species and the morphological characterization of the leaves is necessary for all the taxa involved in the *C. zonatus* complex, since leaf texture has been considered an important diagnostic feature in different identification keys. It is important to continue studying these plants, now using deeper revisionary and population genetics approaches, as a more detailed taxonomical understanding of this group will be critical to be able to decide what to conserve.

4.6. ACKNOWLEDGMENTS

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5. [2]Chapter 2: Anatomical features in *Cryptanthus zonatus* complex and *Orthophytum disjunctum:* a comparative analyses

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Abstract: Anatomical Studies on Bromeliaceae can be useful for taxonomic delimitation and can provide information for phylogenetic inferences, especially in the absence of genetic data. This study surveyed the leaf anatomy of the *Cyptanthus zonatus* complex and *Orthophytum disjunctum* to assess the utility of this character in the delimitation of closely related groups and in the infraspecific level. Leaves of three forms of *C. zonatus* and *O. disjunctum* were collected, and paradermal and transversal sections were made and mounted on permanent slides. The results showed that within the complex *C. zonatus* the pattern, shape and arrangement of the leaf tissues or structures were homogeneous. In the comparative analysis of *O. disjunctum* and *C. zonatus* the leaves anatomical pattern was very similar between these species, but differences were observed like the difference in thickness of the aquíferous parênquima and the presence of ridges also on the adaxial surface in *O. disjunctum*.

Keywords: leaf pattern, taxonomic delimitation, Bromeliaceae

Resumo: Estudos anatômicos em Bromeliaceae podem ser úteis para a delimitação taxonômica, e podem fornecer subsídios para inferências filogenéticas, principalmente na falta de dados genéticos. Nesse estudo procurou-se fazer uma análise da anatomia foliar do complexo *Cyptanthus zonatus* e de *Orthophytum disjunctum* para avaliar a utilidade desses dados na delimitação de grupos próximos e em nível infraespecífico. Folhas das três formas de *C. zonatus* e de *O. disjunctum* foram coletadas, cortes transversais e paradermais foram feitos e montados em lâminas permanentes. Os resultados mostraram que dentro do complexo *C. zonatus* não houve diferenças no padrão, formato e disposição dos tecidos ou de estruturas foliares. Entre *C. zonatus* e *O. disjunctum*, o padrão foliar foi muito similar, mas houve algumas diferenças no que se refere à espessura do parênquima aquífero e presença de ondulações também na superfície adaxial em *O. disjunctum*.

Palavras-chave: Padrão Foliar. Delimitação Taxonômica. Bromeliaceae

5.1. INTRODUCTION

Studies of the leaf anatomy in Bromeliaceae dating from the nineteenth century (FLORES 1974) addressed various aspects (FLORES 1974; ROBINSON 1969; TOMLINSON 1969, HORRES et al. 2007) and revealed that anatomical characters are useful for delimitation of taxa (SOUSA et al. 2005; PROENÇA; SAJO 2007; VERSIEUX; LEME 2007, VERSIEUX et al. 2010). In addition, results from these researches demonstrated that anatomical characters can be synapomorphic and, therefore, can be valuable for the study of the phylogeny of groups (HORRES et al 2007; MONTEIRO; FORZZA; MANTOVANI 2011), mainly because genetic characters available to the family are rare (MONTEIRO; FORZZA; MANTOVANI 2011). Although the literature shows that the leaf structure of the family present certain common patterns regarding the distribution of leaf tissues, variations in this arrangement allowed the differentiation of anatomical groups corresponding to generic groups in many studies (MANTOVANI et al. 2012, HORRES et al. 2007). Moreover, according to (BRAGA 1977), besides contributing to phylogenetic and taxonomic studies, the leaf anatomy can also provide knowledge about the physiology and ecology of these plants.

Despite the importance of these anatomical characters for taxonomic description, research in this area almost always is limited to a few species (TOMLINSON 1969, SCHMITH; BROWN 2004). Therefore the usefulness of these characters in the systematics of many genera are unknown (HORRES et al. 2007; MONTEIRO; FORZZA; MANTOVANI 2011), as is the case for *Cryptanthus* and *Orthophytum*. Regarding the former, only one consistent anatomical study provided a general anatomic description for *Cryptanthus* (RAMÍREZ-MORILLO 1996), however it did not emphasized the variation of anatomical characters among the taxa studied. For *Orthophytum*, only a few studies used anatomic characters in the analysis of species (e.g. VERSIEUX; LEME 2007; FERREIRA; BELLINTANI; DA SILVA 2008). Nevertheless, the published data for these genera allows us to infer that the anatomical characters in *Cryptanthus* may vary within the genus, and that within *Orthophytum* it can vary between species (VERSIEUX; LEME 2007). In addition, *Cryptanthus* and *Orthophytum* are considered sisters groups with differences in floral morphology and chromosome number (RAMÍREZ-MORILLO 1996), and their closed relation is supported by molecular data (SHULTE; HORRES; ZIZKA 2005; SHULTE; BARFUSS; ZIZKA 2009). *Cryptanthus zonatus* complex, on the other hand, is historically ornamental (BAKER 1889) and is composed by three morphotypes (forms): *fuscus*, with red leaf; *viridis*, with green glabrous leaf and *zonatus*, with transversal bands of trichomes (SMITH; DOWNS 1979). For all that reported above, the aim of this work was to provide a more complete analysis of the *Cryptanthus zonatus* complex anatomy and a comparative approach between this complex and the closely related genus *Orthophytum* in order to evaluate the viability of the use of this taxonomic tool for taxonomic delimitation in generic, speciefic and infraspecific level.

5.2. METHODOLOGY

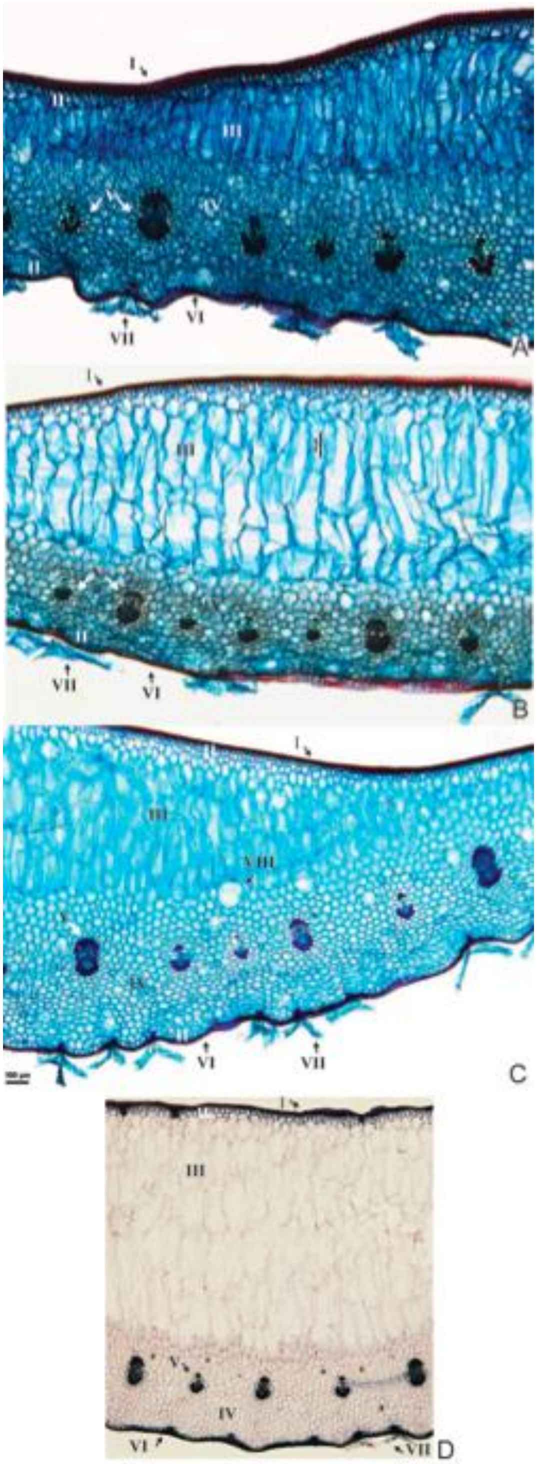
The study was concentrated in areas of Atlantic Forest of the Rio Grande do Norte state at the Parque Estadual das Dunas (PEDN) and Mata Estrela Reserve. The former is an area located in the eastern city of Natal, having geographical coordinate 5 ° 48' S 35 ° 11" W and is the first protected area created in the state. The Private Reserve of Natural Heritage Mata Estrela Senator Antonio Farias (PRNP) 06 º 22 S 35 º 00' W, is located in the municipality of Baía Formosa, and was founded by decree law No. 20/2000, having a total area of 2,039 hectares (BRASIL 2000). Both are the unique areas of known ocurrence of for *C. zonatus* in the Rio Grande do Norte (VERSIEUX; MAGALHÃES; CALVENTE 2013). In addition, samples of *Orthophyum disjunctum* L.B. Smith were made in an area of Caatinga vegetation, at the municipality of Serra de São Bento 6°25’29.3”S; 35°41’57.9W, also in the state of Rio Grande do Norte.

For the analysis, leaves of the three forms of *C. zonatus* (form *fuscus*, form *viridis* and form *zonatus*) and *Orthophyum disjunctum* were collected in field, fixed in 70% ethanol, and transversal and paradermal sections of both leaf surfaces, abaxial and adaxial, were made freehand using a razor blade. To evaluate the composition, distribution and shape of cells and foliar tissues on optical microscope, the sections were subjected to three types of stains: safrablau (safranin and astra blue combined), toluidine blue and methylene blue. After stained, sections were submited to a graded ethanol series (50%, 70% and 100%) and fixed on slides with permanent resin. The slides were observed and photographed under an optical microscope Nikon “Eclipse Ni” with coupled camera “Nikon DS-Ri”. Additional analyses of *C. zonatus* specimen were carried in scanning electron microscopy (SEM). For SEM analysis paradermal sections were preserved in 70% ethanol and then were observed in electronic microscopic Hitachi TM 3000.

5.3. RESULTS

In transverse section, the leaf of the three *C. zonatus* forms (fig. 1) exhibited an adaxial epidermis formed by one layer of rectangular cells with well thickened and lignified anticlinal and periclinal internal walls, leaving the lumen with a somewhat triangular shape, and the external periclinal walls with small irregular projections (fig. 2 B-D). Adjacent to the adaxial epidermis there is a hypodermis presenting 2-3 layers of rounded cells with irregular size and thickened walls (fig. 2 B-D). An aquiferous parenchyma with expanded, rounded to elongated cells occurs below the adaxial hypodermis and is increasingly thicker towards the base of the leaf, varying from absence (close to the leaf apex) to approximately four layers, reaching an maximum of 50% of the leaf thickness (fig. 1). Inside the aquiferous parenchyma mucilage cells or channels may occur (fig. 1 C). There is an abrupt transition from the aquiferous parenchyma to the chlorenchyma, where collateral vascular bundles appear side by side (figs. 1, 2 H). Adjacent to chlorenchyma towards the abaxial surface there is a hypodermis composed by one layer of rounded cells which may present regular or irregular size depending on the specimen (figs. 1, 2 E-G). The abaxial hypodermis cells present thickened walls and in some specimen the periclinal external walls are thicker than the anticlinal and internal periclinal walls (fig. 2G). Below this hypodermis lies the abaxial epidermis with one layer of rectangular cells with thickened anticlinal and internal periclinal walls and external periclinal walls narrowly thickened (fig. 2 E-G). The central region of the abaxial epidermis cells (corresponding to the cell protoplasm) may be projected outwards forming a rounded convex protuberance. The abaxial epidermis presents an undulate curvature, marked by the occurrence of trichomes, while the adaxial side is plane (fig. 1). Stomata occur only in the abaxial epidermis and in the same level of the ordinary epidermis cells (fig. 2 J). Hypostomatic chambers occur bellow stomata and reach the interior of the mesophyll (fig. 2 J).

Figure 1.Transversal section of the leaf of *Cryptanthus zonatus* f. *fuscus* (A), C. *zonatus* f. *zonatus* (B), *C. zonatus* f. *viridis* (C) and *Orthophytum disjunctum* (D). I. Adaxial epidermis, II. Hypodermis. III. Aquiferous parenchyma. IV. Chlorenchyma. V. Vascular bundles. VI. Abaxial epidermis. VII. Trichome. VIII. Mucilage channel.

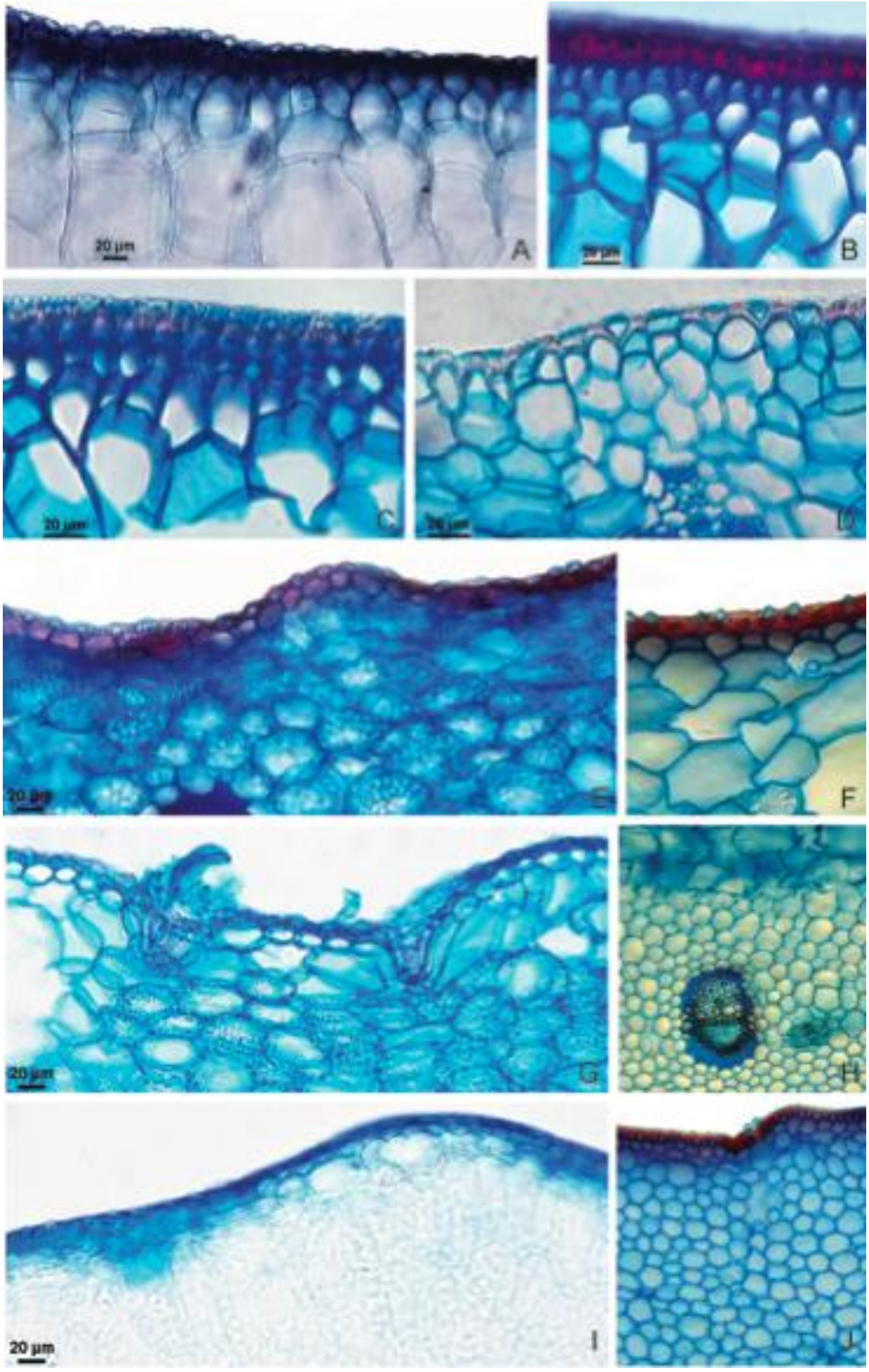


Source: optical microscope Nikon "Eclipse Ni" with coupled camera "Nikon DS-Ri″

In paradermal sections the adaxial epidermis cells are squared to rectangular, with sinuous thickened anticlinal walls and very reduced lumen (figs. 3 B-G, 4). The abaxial epidermis cells are rectangular-elongated with narrower thickened anticlinal walls (figs. 3 B-G, 4). Globular silica bodies can be observed in all abaxial epidermis cells (figs. 3 B-G, 4). The tetracytic stomata are dispersed irregularly throughout the abaxial epidermis (fig. 4 G, I). Peltate trichomes are also dispersed irregularly throughout the abaxial side of the leaf, occurring isolated or in small groups and scarcely and in smaller density are also found in the adaxial side of the leaf in *C. zonatus* f. *zonatus* (figs. 3 E, 4 B-E, H). Trichomes occur equally in both sides of the leaf only in the leaves apexes of the three *C. zonatus* forms. Each trichome presented a stalk formed of two cells in all of *C. zonatus* forms (fig. 3 A).

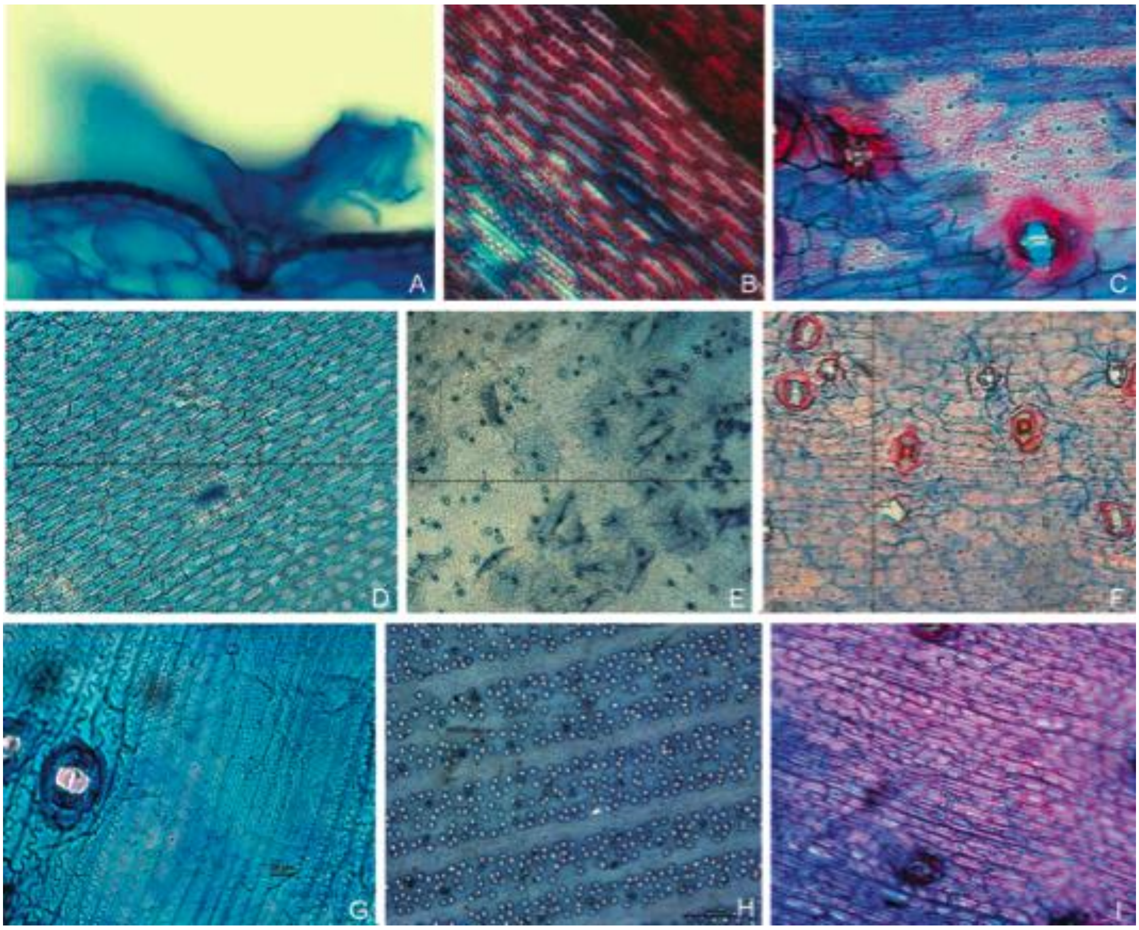
Orthophytum disjunctum showed an adaxial epidermis composed of cells with thickened walls (fig, 2 A). Just bellow this epidermis there is a hypodermis comprising 1-2 layers of cells larger than the epidermis cells (fig. 1 D). The aquiferous parenchyma is quite developed occupying approximately 70% of the thickness of the leaf, and it is formed by elongated cells (fig. 1 D). Bellow there is a chlorenchyma with rounded cells that occupies approximately 30% of the thickness of the leaf blade (fig. 1 D). Just bellow the chlorenchyma occurs a hypodermis formed by one layer of rounded cells with moderate thickened walls (fig. 1 D). The abaxial epidermis consists of one layer of cells with thickened cutinized walls and reduced lumen (fig. 1 I). The tetracytic stomata were restricted to the abaxial surface where they are arranged in longitudinal rows and are located at the same level of the other epidermal cells (fig. 3 H-I). In both leaf surfaces, the cells are rectangular-shaped with sinuous walls and present trichomes. Both epidermis exhibited a subtle irregular curvature. Two cells form the stalk of trichomes. Collateral vascular bundles are placed laterally in a row throughout the clorenchyma and display outer and inner sheaths composed by one layer of cells.

Figure 2. Transversal sections of the leaf of *O. disjunctum* (A, I), *C. zonatus* f. *zonatus* (B, E), *C. zonatus* f. *fuscus* (C, G), *C. zonatus* f. *viridis* (D, F, J, H). A-D. Adaxial epidermis. E-G, I. Abaxial epidermis. H. Detail of vascular bundle. J. Detail of stomata with substomatal chamber.



Source: Optical microscope Nikon "Eclipse Ni" with coupled camera "Nikon DS-Ri"

Figure 3. A. Trichome of *C. zonatus* f. *fuscus*. B-I. Paradermal sections. *C. zonatus* f. *fuscus* (B-C). Adaxial (B), Abaxial (C). *C. zonatus* f. *viridis* (D-E, F). Adaxial (D), Abaxial (E,F). Abaxial epidermis of *C. zonatus* f. *zonatus* (G). *O. disjunctum* (H-I). Abaxial (H), Adaxial (I).



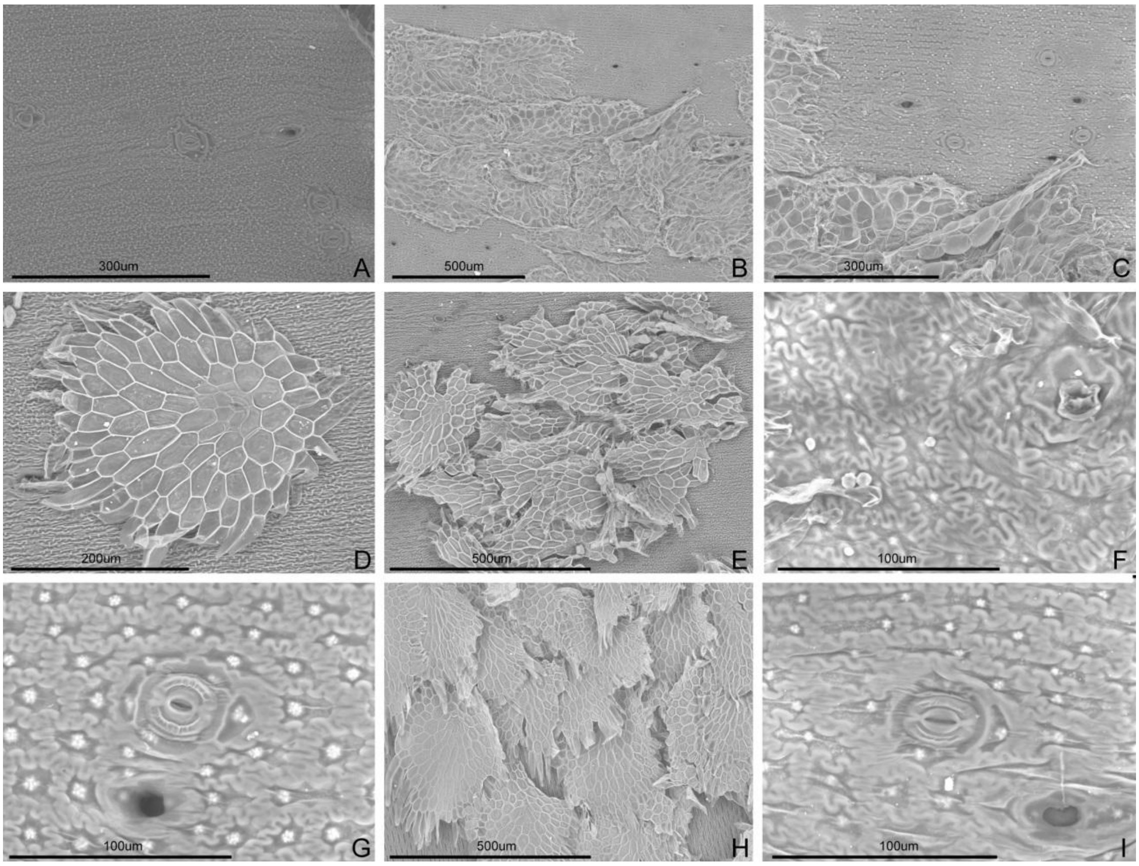
Source: Optical microscope Nikon "Eclipse Ni" with coupled camera "Nikon DS-Ri"

5.4. DISCUSSION

The leaves of *C. zonatus* and *O. disjunctum* showed a pattern that conforms to the general pattern presented to the family Bromeliaceae (TOMLINSON 1969; SCATENA; SEGECIN 2005; PROENÇA; SAJO 2007; VERSIEUX et al. 2010; MONTEIRO; FORZZA; MANTOVANI 2011; MANTOVANI et al. 2012) and the genus *Cryptanthus* (RAMÍREZ-MORILLO 1996). The anatomical pattern is more similar to the type IV defined by

(HORRES et al. 2007), although according to the author, this type presents air channels well developed, which was not observed here.

Figure 4. Eletronic Micrographs of epidermis of *C. zonatus* f. *fuscus* (F), *C. zonatus* f. *viridis* (B, C, I), *C. zonatus* f. *zonatus* (A, D, E. H).



Source: Electronic microscopic Hitachi TM 3000

In the tree forms of *C. zonatus*, the adaxial and abaxial epidermis are different. Among the observed differences are: the size of cells, which are bigger in the adaxial epidermis in comparison with the abaxial epidermis cells; and the shape of the cellular lumen in transversal section, which is triangular in the adaxial epidermis and rounded and projected in the abaxial epidermis. In addition, the lumen of adaxial epidermis cells is smaller than the lumen observed in the abaxial epidermis cells, which can result due to the wider thickening of the adaxial cells walls. The reduced lumen and the thickened cells walls are frequently observed in Bromeliaceae (FLORES 1975, SCATENA; SEGECIN 2005; PROENÇA; SAJO 2007;VERSIEUX et al. 2010; MONTEIRO; FORZZA; MANTOVANI 2011; MANTOVANI et al. 2012) and represent xeromorphic characters (SOUSA; ESTELITA; WANDERLEY.

2005, HABERLANT 1914), as the thickening of epidermis cells walls would prevent the water loss (VERSIEUX et al. 2010). Another difference observed was the absence of silica bodies in the adaxial epidermis. The presence of silica crystals is very commonly observed in Bromeliaceae and may be associated to light reflection (preventing heat rise and increased water loss) (KRAUSS 1949) and a protection against herbivores (ZANENGA-GODOY; COSTA 2003). The number of hypodermis layers also differed between both leaf sides, as the adaxial side is formed by 2-3 layers and the abaxial side presented only one layer. The presence of the hypodermis is frequently observed for Bromeliaceae (AYOAMA; SAJO 2003; SOUSA; ESTELITA; WANDERLEY 2005; VERSIEUX et al. 2010). Both epidermis also varied in curvature, as the adaxial epidermis is plane while the abaxial epidermis is slightly undulate. The curvature of the abaxial epidermis is associated with the distribution of trichomes, which are found mostly in the abaxial epidermis.

The epidermis cells did not vary in shape, presenting the rectangular form, which was consistent with the literature findings for Bromeliaceae (SCATENA; SEGECIN 2005). Both sides of the leaf exhibited epidermis cells with sinuous walls, also a common finding for all Bromeliaceae species, even as far as species from different Bromeliaceae subfamilies (TOMLINSON 1969, FLORES 1975; SOUSA; ESTELITA; WANDERLEY 2005; PROENÇA; SAJO 2007).

The leaves of the three forms are classified as hypostomatic, with stomata located in the same level of the ordinary abaxial epidermal cells, as commonly found for other Bromeliaceae species (SCATENA; SEGECIN 2005, SOUSA et al. 2005, PROENÇA; SAJO 2007). *Orthophytum disjunctum* also presented the same features. Both species presented tetracytic stomata (CUTLER; BOTHA; STEVENSON 2011). Hypostomatic leafs are associated with mesophytic environments (CUTLER; BOTHA; STEVENSON 2011). The presence of an expanded aquiferous parenchyma is considered a xeromorphic character (SOUSA; ESTELITA; WANDERLEY. 2005; HABERLANT 1914) and is also frequently observed for Bromeliaceae (FLORES 1975; AYOAMA; SAJO 2003, SOUSA et al. 2005, SCATENA; SEGECIN 2005, PROENÇA; SAJO 2007; VERSIEUX et al. 2010, MONTEIRO; FORZZA; MANTOVANI 2011; MANTOVANI et al. 2012). The fact that *O. disjunctum* presented a bigger proportion covered by the aquiferous parenchyma indicates that it may tolerate harsher xeric conditions.

The presence of trichomes, although generally related to the reduction of water loss due to protection against sunlight and transpiration (FLORES 1975) is referred to as a synapomorphy of the family (BENZING; GIVNISH; BERMUDES 1985; BENZING 2000). Also with respect to the trichomes, the number of stalk cells, which in the case of the species studied here was two cells, is regarded as one derived character within the subfamily Bromelioideae (TONLINSON 1969). According to (TONLINSON 1969), the same number of stalk cells is also found in other species of the genus *Cryptanthus*. (VERSIEUX; LEME 2007) found the same number of stalk cells in other species of *Orthophytum*, but this character could vary within this genus. The lack of extrafascicular fiber bundles is common in Bromeliaceae, being observed only in Bromelioideae and its presence is considered a derived character (PROENÇA; SAJO 2007; MONTEIRO; FORZZA; MANTOVANI 2011).

In general, the anatomical pattern found for *O. disjunctum* is very similar for what was observed in *C. zonatus*. However, *O. disjunctum* differed in the more succulent leafs, a larger aquiferous parênquima, in the shape of the epidermal cells and in the undulate curvature exhibited by both epidermis (in *C. zonatus* only the adaxial surface is undulate). Despite the fact that *O. disjunctum* presents trichomes in both leafs sides, this character is not relevant to distinguish both species as *C. zonatus* f. *zonatus* also presented this character. Maybe the higher predominance of trichomes in this species may be related to a more xeric environment occupied by *O. disjunctum*. *Cryptanthus zonatus* is more common in the Atlantic rain forest in more mesophytic environment while *O. disjunctum* can tolerate the Caatinga, a more arid environment. Furthemore, *O. disjunctum* is saxicolous; occuring in a more extreme open habitat over rocks what contrasts with *Cryptanthus zonatus*, which is terrestrial occurring in the more humid forests understories (VERSIEUX; MAGALHÃES; CALVENTE 2013). Nevertheless, the similarities found between both species can be expected as both genera are very close phylogenetically (SHULTE; HORRES; ZIZKA 2005; SHULTE; BARFUSS; ZIZKA 2009) and probably share several common ancestral conditions.

5.5. CONCLUSION

The three morphotypes of *C. zonatus* showed no significant anatomical differences that could be used for taxonomy. Despite the observed differences on trichome distribution, this character is not consistent, since some species may present or not trichomes on both leaf surfaces (RAMÍREZ-MORILLO 1996). Thus, the anatomical characters were not taxonomically significant to separate the three morphotypes

O. disjunctum presents a leaf pattern very similar to *C. zonatus* complex, but differed by the stomata arranged in rows on the abaxial surface of the leaf and by the ridges observed in the adaxial side while in *C. zonatus* the adaxial surface is plane. In addition, *O. disjuncum* presented a wider proportion of the leaf covered by the aquiferous parenchyma. Thus the anatomical features were useful to distinguish the two species.

5.6. ACKNOWLEDGMENTS

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6. [3]Chapter 3: Notes on the phenology, floral biology and morphology of the *Cryptanthus zonatus* complex in Rio Grande do Norte, Brazil

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Abstract:

In this chapter we conduct a preliminary investigation of the phenology, floral biology and floral morphology of *Cryptanthus zonatus*. Since it is a species of complex taxonomy that presents phenotypic variation, having three morphotypes, we sought to evaluate differences within the *C. zonatus* complex find here in the state of Rio Grande do Norte, Brazil. During this study we conducted monthly visits to the field, collected nectar for the mesure of concentration and made measurements of floral parts. The results show that all three forms overlap in flowering periods, and data from the literature along with data obtained here indicate a similarity in nectar volume. Furthermore, there was great similarity with respect to floral morphology, the results not being conclusive for consistent taxonomic delimitation of the three forms and indicating the need of further long-term studies.

Key-words: Phenotypic Variation. Timing of Flowering. Nectar. Flower Parts.

Resumo:

Nesse capítulo foi feita uma investigação preliminar da fenologia, biologia floral e morfologia floral de *Cryptanthus zonatus*. Visto que é uma espécie de taxonomia complexa que apresenta variação fenotípica, possuindo três morfotipos, procurou-se avaliar diferenças dentro do complexo *C. zonatus* encontradas aqui no estado do Rio Grande do Norte, Brasil. Durante o estudo foram conduzidas visitas mensais a campo, o néctar foi coletado para a mensuração da concentração e as peças florais foram medidas. Os resultados mostram que as três formas encontradas apresentam sobreposição nos períodos de floração, e os dados obtidos na literatura juntamente com os dados obtidos aqui apontam uma semelhança no volume de néctar. Além disso, houve grande similaridade com relação à morfologia floral, não sendo os resultados conclusivos para a delimitação taxonômica consistente dessas três formas e indicando a necessidade de estudos posteriores de longo prazo.

Palavras – chave: Variação Fenotípica. Período de Floração. Néctar. Peças Florais.

6.1. INTRODUCTION

According to the U.S. / IBP Phenology Committee, the term phenology is defined as the study of the timing of recurring biological events, the relationship of these events to biotic and abiotic factors, and the interrelation among phases of the same species or different species (see PUPPI 2007). Phenologycal studies have been addressed by several authors (e.g. BORCHERT 1983, RATHCKE; LACEY 1985, KOCHMER; HANDEL 1986, VAN SCHAIK; TERBORGH; WRIGHT 1993, TALORA; MORELLATO 2000, BARROS; GRAY; DIAZ-CASTELAZO 2001, SAKAI 2001, BENCKE; MORELLATO 2002, D'EÇA-NEVES; MORELLATO 2004, MACHADO; SEMIR 2006, SHERRY et al. 2007, CHUINE 2010, CARVALHO JÚNIOR; MELO; MARTINS 2011), due to its importance for reproductive success (SAKAI 2001, ELZINGA et al. 2007). As the phenological pattern is a result of the intervention of biotic and abiotic factors (OLLERTON; LACK 1992, VAN SCHAIK; TERBORGH; WRIGHT 1993, SAKAI 2001, ELZINGA et al. 2007), the phenology is one adaptation for these factors, and in this way, is considered a key feature to determine the ecological niche (DONOHUE 2005), and may also serve as a driving force of speciation processes in plants (NEWSTROM; FRANKIE; BAKER 1994, ELZINGA et al. 2007). In tropical forests, phenology is much more complex than in temperate regions (GENTRY 1974, NEWSTROM; FRANKIE 1994). Among the phenological phases, the study of the flowering period is extremely important due to the close relationship between the pollinator and the flowering peak in the population and therefore relevant to understand the strategies used by the plant to achieve a higher reproductive success (ELZINGA et al. 2007).

Also, regarding plant-pollinator interaction, the literature shows a tendency to relate floral features or signs (collectively treated as “pollination syndromes”) such as the shape of the flower, the color, the number of flowers per inflorescence; the pattern of odor emission, the amount and time of nectar production to specific pollinator or group of pollinators (KINGSTON;QUILLAN 2000; CANELA; SAZIMA 2003, 2005; FENSTER 2005, MACHADO et al. 2007). The relation between plants and their pollinator has been target by many authors (e.g. BAWA 1990; WASER 1996; FENSTER 2005; KINGSTON; QUILLAN 2000) and are complex (Waser 1996). Although the pollination syndrome concept is widely used (WASER 1996), researches show that a more generalized grid of pollinators is common (FENSTER 2005), thus the pollinator syndrome concept not always predict the correct pollinator (KINGSTON;QUILLAN 2000).

But although more than one group of pollinators could visit the plant, their effectiveness differs (FENSTER 2005). The kind of pollinator, on another hand, can influence the matting system (VOGLER; KALISZ 1998). The matting systems could be also influenced by exhibition of floral traits (BRUNET; ECKERT, 1998), size and flower daily production (GOODWILLIE et al. 2010).

Phenological studies show that the community or population of Bromeliaceae has a sequential pattern of flowering (ARAÚJO; FISCHER; SAZIMA 1994, MACHADO; SEMIR 2006, SIQUEIRA-FILHO; LEME 2006, VOSGUERITCHIAN; BUZATO 2006, MARQUES; LEMOS FILHO 2008). This pattern allows a continuous supply of resources to the pollinators (ARAÚJO; FISCHER; SAZIMA 1994), which are mainly hummingbirds (SIQUEIRA-FILHO; MACHADO 2001, CANELA; SAZIMA 2003, 2005; KAEHLER; VARASSIN; GOLDENBERG 2005; SIQUEIRA-FILHO; LEME 2006; MACHADO; SEMIR 2006, VOSGUERITCHIAN; BUZATO 2006; BUZZATO; SAZIMA; SAZIMA 2000.). Bromeliads are distinct from most plant families by having vertebrate pollination predominating over entomophily (SAZIMA; VOGEL; SAZIMA 1989).

Cryptanthus zonatus is a succulent terrestrial bromeliad. It is a polymorphic species belonging to the subfamily Bromelioideae (SMITH; DOWNS 1979) presenting three forms (RAMÍREZ-MORILLO 1996) or color morphs. It occurs in sandy soils in the Atlantic rain forest and is an endemic species from Brazil threatened of extinction (FORZZA et al. 2013a, 2013b). *Cryptanthus* is popularly known as earth-stars and, particularly *C. zonatus* is world-wide cultivated (VERSIEUX; MAGALHÃES; CALVENTE 2013). In Rio Grande do Norte, sympatric specimens of these color morphs (forms) may be found inside the Atlantic forest domain (VERSIEUX; MAGALHÃES; CALVENTE 2013). As the segregation of such color morphs in distinct taxonomic entities has confused taxonomists, in this study we conducted a preliminary analysis of floral morphology and floral biology, as well as observations of the phenology of *Cryptanthus zonatus* (Vis.) Beer complex in order better understand them.

6.2. METHODOLOGY

Study area

The phenological studies were carried out in areas of Atlantic Forest inside the Parque Estadual Dunas de Natal Jornalista Luiz Maria Alves (PEDN) 5°49’12”S, 35°11’16” and Private Reserve of Natural Patrimony Mata Estrela (PRME) 06º22’10”S 35º00’28”W, Rio Grande do Norte State, northeastern Brazil. The former is an urban park located inside the city of Natal. The Private Reserve of Mata Estrela is located in the municipality of Baía Formosa (Figure 1).

Both the PEDN and the PRME are covered by Atlantic Forest and the coastal sand dunes shrubby vegetation. In PEDN trails that were monitored were: Ubaia doce, Peroba, Perobinha, Três Marias and Pau-Brasil. In PRME the trails were Coca-cola, Pagão, Gameleira e Pau-Ferro. By visual observation the populations were more fragmented in PEDN if compared to the long mats found in PRME. In both areas the *zonatus* form was apparently rarer.

Phenology

For analysis of phenological pattern, we made monthly visits to both locations during one year (from April of 2012 to April of 2013), using the method of trails (D’EÇA-NEVES; MORELATO 2004), without a pre-established number of individuals to be monitored. Pre-established trails were explored and qualitative analyzes (presence or absence) (BENCK; MORELLATO 2002) of flowering and fruiting phenophases were made in population of the three forms. Additionally, herbarium data (from SpeciesLink, UFPE herbarium, 2 specimens with flowers) were analyzed to check for the presence of flowers and the date they were collected.

Floral biology

Observations of the populations of the three forms of *C. zonatus* were made in the field during the flowering period: the number of open flowers per day; the time of anthesis and senescence of the flower. In addition, the volume and concentration of solutes in nectar were evaluated in the field in the morning using a graduated micro-syringe (Hamilton) 25µl and a pocket refractometer, 0-32% Brix. A t-test was conducted in Excell software to compare mean values for nectar production. Additional observations were made in cultivated specimens, particularly for checking the right time of anthesis.

Floral morphology

For the floral morphology analysis, flowers of the three forms were collected in the PRME and stored in 70% ethanol (N = 3 for *viridis*, 3 for *fuscus* and 4 *zonatus*). Then they were analyzed under a stereomicroscope and measurements of floral parts were made with a caliper. Finally, the morphological characterization was performed using a specialized glossary (BEENTJE 2010) and flowers were illustrated by a professional botanical artist. For the Scanning Electron Microscopy stigma and anthers of the three forms were collected in field. The anthers were stored on filter paper inside a vial containing silica gel and the stigmas were fixed in 70% ethanol. Subsequently the anthers and stigmas were observed by scanning electron microscopy in a digital scanning microscope Hitachi TM 3000 at the Electronic Microscopy Laboratory, UFRN.

Floral visitor

Due to the limited blooming of individuals found in the field, observations of visitors were restricted to 5 hours and carried out only in Mata Estrela and only for the green color morph (*viridis* form). The visitor was photographed with a digital camera, and the specimen was collected and later identified with the help of a taxonomist (see acknowledgments).

6.3. RESULTS AND DISCUSSION

Phenology

The form *fuscus* presented the flowering period in February, April and October, and fruiting in February, July and August. The *viridis* form presented flowering period in the months of March and April, and fruiting in August. The form *zonatus* presented flowering period during the months of March and April. Analyzing these results we can see that in April there is an overlap of the flowering period of the three forms (Box 1).

Box 1. Phenological phases of the three forms of *C. zonatus* in Rio Grande do Norte (RN), Brazil, beginning in April 2012. Black squares indicate months with flowering and pale grey indicates fruiting. Blue indicates the rainy season. Observations conducted in eastern RN, in Parque das Dunas and Mata Estrela.

	M	A	M	J	J	A	S	O	N	D	J	F	M	A
C. zonatus f. *fuscus*		■						■				■		■
					▒	▒						▒		
C. zonatus f. *viridis*													■	■
						▒								
C. zonatus f. *zonatus*													■	■
Rainy season			🟦	🟦	🟦	🟦	🟦							

This is in accordance with data from the literature, showing the months of March and April as the reproductive period of this species (SIQUEIRA-FILHO; LEME 2006) in the state of Pernambuco. The flowering period seen in the field is also supported by two specimens found in on-line hebarium collections that flowered in the months of April and May. The slight asynchronism between the forms *viridis* and *zonatus* related to *fuscus* can be considered a variation of the population, since according to (HENDRY; DAY 2005) and (ELZINGA et al. 2007), populations are variable with respect to their reproductive periods. According to data from (EMPARN 2013), the year of 2012 was atypical for presenting a rainfall below the average but apparently there is a relationship between period of more rainfall and fruiting.

It is important to keep monitoring the species on the long term, to allow a better understanding of its phenological pattern, but if we consider the data available in the herbaria together with the data collected in present work, we can consider *C. zonatus* f. *fuscus* as subanual according to (NEWSTROM; FRANKIE; BAKER 1994) classification, since there are two flowering periods. And the other forms may be annual, although more years of observation should be conducted.

During flowering period, the number of opened flowers per day/per rosette varied from one to two in *zonatus* and *fuscus* forms and from one to four in the *viridis* form. The number of open flowers per day fit in the steady-state of (GENTRY 1974) and also is in agreement with findings in the literature for this species (SIQUEIRA-FILHO; MACHADO 2001) and for *C. dianae*, which is another species of the genus (SIQUEIRA-FILHO 2003). In the population of PDD, were found only three individuals with fruits while in Baía Formosa only one individual with fruits was observed, and all these specimens belonged solely to the *viridis* or *fuscus* forms.

Floral biology

The flowers of the three forms can be either staminate (or incomplete) or bissexual (complete), which is characteristic of *Cryptanthus* (RAMÍREZ-MORILLO 1996, BENZING 2000). At our first observation in the early morning, between 5:30 a.m. to 6:00 a.m, flowers of the three forms were already openned. But cultivated specimen of *fuscus* form openned its flowers by this same time, 5:30-6:00 h (personal observation) and the literature also indicated a diurnal anthesis for other taxa belonging to *Cryptanthus* (SIQUEIRA-FILHO 2003; SIQUEIRA-FILHO; LEME 2006). The flowers last for a single day, withering at the late afternoon, as observed in the field and also in cultivate material. Such short life-span is in accordance with literature reports (RAMÍREZ-MORILLO 1996). The diurnal anthesis in turn is frequently reported for the family in general (e.g. CANELA; SAZIMA 2003, 2005, MACHADO; SEMIR 2006), and is associated to the main pollinators found in the bromeliads, such as hummingbirds, insects, although bat pollination may be found in some genera, particularly in Tillandsioideae.

The observations made so far showed that the forms *viridis* and *fuscus* were visited by bees of the genus *Trigona*, Tribe Meliponini (Figure 2). In the *zonatus* form we did not conducted focal observations due to lack of flowers. This result is in agreement with the literature, which indicates melittophily syndrome to *Cryptanthus* (SIQUEIRA-FILHO 2003), and also to the complex *C. zonatus* (SIQUEIRA-FILHO; LEME 2006). Insects are also suggested as possible pollinators by (RAMÍREZ-MORILLO 1996). The Tribe Meliponini has also been reported for other species of Bromeliaceae (NARA; WEBBER 2002). This floral visitor were observed in the morning, and presented a pillager behavior, landing over the anthers and collecting pollen, not getting in contact the stigma. This behavior is in agreement with the literature for this type of bee pollinator (NARA; WEBBER 2002, ODA; ODA 2007). Although the pollinator has not been observed in the form *zonatus* the melittophily has been reported previously for *C. zonatus* form (SIQUEIRA-FILHO; LEME 2006), then is more likely that the bee *Trigona* is also the floral visitant of *zonatus* form, once that this bee genus is common in the Neotropic region (WILLE 1993). According to (HINGSTON; MCQUILLAN 2000) the melittophily syndrome (sensu FAEGRI; PIJL 1979) is characterized by yellow or blue and zygomorphic flowers, while sphecophily (pollinated by wasp), cantharophily (pollinated by beetles) and myophily (pollination by flies) show colorless and actinomorphic flowers. (BAWA 1990) mentions that the flowers pollinated by bees can also be white, light or green, similar to what is seen in the *C. zonatus* complex flower.

For *Cryptanthus zonatus* f. *fuscus*, the mean of nectar volume was 8 µl per flower (±2.91 µl; N=4) and the average concentration of nectar was 11.5% (± 6.60%, N = 4). The form *viridis* had a mean volume of nectar of 7.22 µl (±3.23 µl; N=9), and an average concentration of nectar 17.89% (± 6.77%, N = 9). The t-test for comparison of nectar volume between the two forms *fuscus* and *viridis* was t = -0.68892136 and the concentration of nectar between the two forms was t = 0.142347549, not being considered significant. So the mean volume and concentration of nectar were similar between the two forms. The volume of nectar found in the forms *fuscus* and *viridis* confirms results previously found for *C. dianae* Leme (SIQUEIRA-FILHO 2003).

Table 1. Nectar concentration and volume in *C. zonatus* f. *viridis* and *C. zonatus* f. *fuscus*.

	C. zonatus f. *viridis*		*C. zonatus* f. *fuscus*	
	Nectar volume	Concentration of nectar	Nectar volume	Concentration of nectar
	5µl	30%	12µl	7%
	3µl	20%	7,5µl	21%
	10µl	17%	7,5µl	11%
	2,5µl	8%	5µl	7%
	10µl	13%		
	5,5µl	22%		
	8,5µl	21%		
	10,5µl	20%		
	10µl	10%		
Average	7.22 µl	17.89%	8 µl	11.5%
Standard deviation	3.23 µl	6.77%,	2.91 µl	6.60%

The only nectar data available in the literature for *C. zonatus* was presented by (SIQUEIRA-FILHO; MACHADO 2001), where they reported that a concentration of nectar is 28% and a volume of 10µl. Despite here the volume of nectar was not analyzed in the form *zonatus*, our findings for volume corroborate the results found by these authors. But the concentration of nectar in the forms *fuscus* and *viridis* observed here was lower than previously reported. Thus, further measurements should be taken to a more robust analysis.

The population of *fuscus* and *viridis* forms at Mata Estrela seems larger than that of *zonatus* form. The three forms showed stolons, which means that they reproduce vegetatively. Vegetative reproduction, on the other hand, it is quite common in Bromeliaceae (BENZING 2000). Given the low number of individuals available with flowers, even for our focal observations, we suggest that new studies should focus on the vegetative growth strategies employed by this species.

Floral morphology

Basically the flowers do not vary much in their morphology. Here we present a basic description of each morph (Box 2):

The flowers of *C. zonatus* f. *fuscus* are characterized as follows: floral bracts somewhat smaller than the sepal, with membranous texture, boat-shaped, oval-lanceolate, apex acute-serrate, carinate, measuring 2-2.3 cm long and 0.5-0.6 cm wide. Flower 4.6-5.5 cm long, sepals 2-2.6 cm long, apex acute and smooth. And petals obovate-lanceolate with calyx free lobes measuring 3.1 cm long, with apex acute and apiculate-sub-rounded. Six stamens partially adnate with the corolla, with a white filament with the free part measuring 1.1 cm long and yellow anther 0.2-0.3 cm length, sagittate dorsifixed along the base with longitudinal dehiscence. Stigma trifid. Another flower *C. zonatus* f. *fuscus* also collected from the Baía Formosa, the floral bracts showed smaller size, approximately half the size of sepals.

The flowers of *C. zonatus* f. *viridis* have the floral bracts whitish or brownish, smaller than the sepals, equaling ¾ of the sepals, and membranous, canaliculate 1.5-1.8 cm long and 0.5 cm wide, strongly carinate, oval-lanceolate, apex acute-acuminate and serrate. The sepals are green hyaline canaliculate, 2.1 cm long, highly connate (± ¾), with lobes measuring 0.7 cm long, apex acute-acuminate, smooth. Flowers, white, trimerous measuring 4 cm long, petals 3.5 cm, lobes 2.8 cm long, apex obtuse, apiculate. Petals obovate-lanceolate. Six stamens with white filaments and free part measuring 1.2 cm in length. Anthers yellowish measuring 0.2-0.3 cm length with longitudinal opening and dorsifixed along the sagittate base. Stigma trifid white. Ovary globular and placentation axial restricted to the top of the locule.

The flowers of the individuals of *C. zonatus* f. *zonatus* collected at Mata Estrela in Baía Formosa, presented: white flowers, ranging from 4.6 to 5.5 cm in length, petals white, obovate- lanceolate, shortly connate. The size of the sepals and floral bracts also varied between 1.5-2 cm long and 2-2.2 x 0.6 cm, respectively. Petals 3.4-4.5 cm long. The filament presented the free portion measuring 1.2 cm. The anther is yellowish and dorsifixed along the sagitated base showed length of 0.3 cm with longitudinal opening. Floral bracts oval-lancelate with 2-2.4 cm long and 0.5-0.6 cm wide, carinate with apex acute-acuminate and serrate, white-greenish with brownish apex.

Box 2. Floral morphology comparison among the three color morphs found in RN.

Color morphs	Flower Length	Sepal Length	Petal Length	Floral Bract Length x Width
C. zonatus f. *fuscus* (N=4)	4.6-5.5 cm	2-2.6 cm	3.7-4.5 cm	2-2.3 x 0.5-0.6 cm
C. zonatus f. *viridis* (N=3)	3.6-4 cm	1.7 cm	3.5 cm	1.5-1.8 x 0.5 cm
C. zonatus f. *zonatus* (N=4)	4.6-5.5 cm	1.5-2 cm	3.4-4.5 cm	2-2.4 x 0.5-0.6 cm

The flower morphology of three individuals proved to be very similar, although some variation in flower length and floral bract size was detected for f. *viridis* (Box 2), but for this form we measured the lower number of samples. We observed that both forms *viridis* and *fuscus* are visited by the same species of bee (Figure 2), and that these forms grow together. The morphology of the stigma was also very similar (Figures 2, 3).

(RAMÍREZ-MORILLO 1996) and (SIQUEIRA-FILHO; LEME 2006) indicate morphological affinity of *C. zonatus* with other species of the genus *Cryptanthus* as *C. dianae*, *C. burle-maxii* Leme and *C. fosterianus* L.B. Sm. In view of these results, and considering the great similarity among the colormorphs and the species cited above, as well as their phenotipic variation (VERSIEUX; MAGALHÃES; CALVENTE Chapter 1), we suggest a deeper revision of this group, involving molecular and morphological data as well as extensive fieldwork.

6.4. CONCLUSIONS

We concluded that the three forms showed very similar floral morphology and that the specimens collected in Rio Grande do Norte fit in *C. zonatus* concept. The overlap of phenology and similarity in flower parts (such as stigma, analyzed by electron microscopy) also suggest that the color morphs are phenotypic variation. The causes os such variation may be related to several factors that should be investigated in more detail in the future.

6.5. FUTURE DIRECTIONS AND SUGGESTIONS

This work is still preliminary and most of our analysis suffered from the limited number of flowers available. We suggest as future approaches: (1) to keep the phenological study that should be extended for more years to securely indicate the flowering pattern (NEWSTROM & FRANKIE 1994) and more frequent visits, given the short life-span of *C. zonatus* flowers (2) mark and observe a larger number of flowers (3) proceed on a reproductive analysis of the color morphs with controlled crosses and investigation of the fruit set and seed viability (4) use genetic markers to understand the phenotypic variability in the complex.

6.6. ACKNOWLEDGMENT

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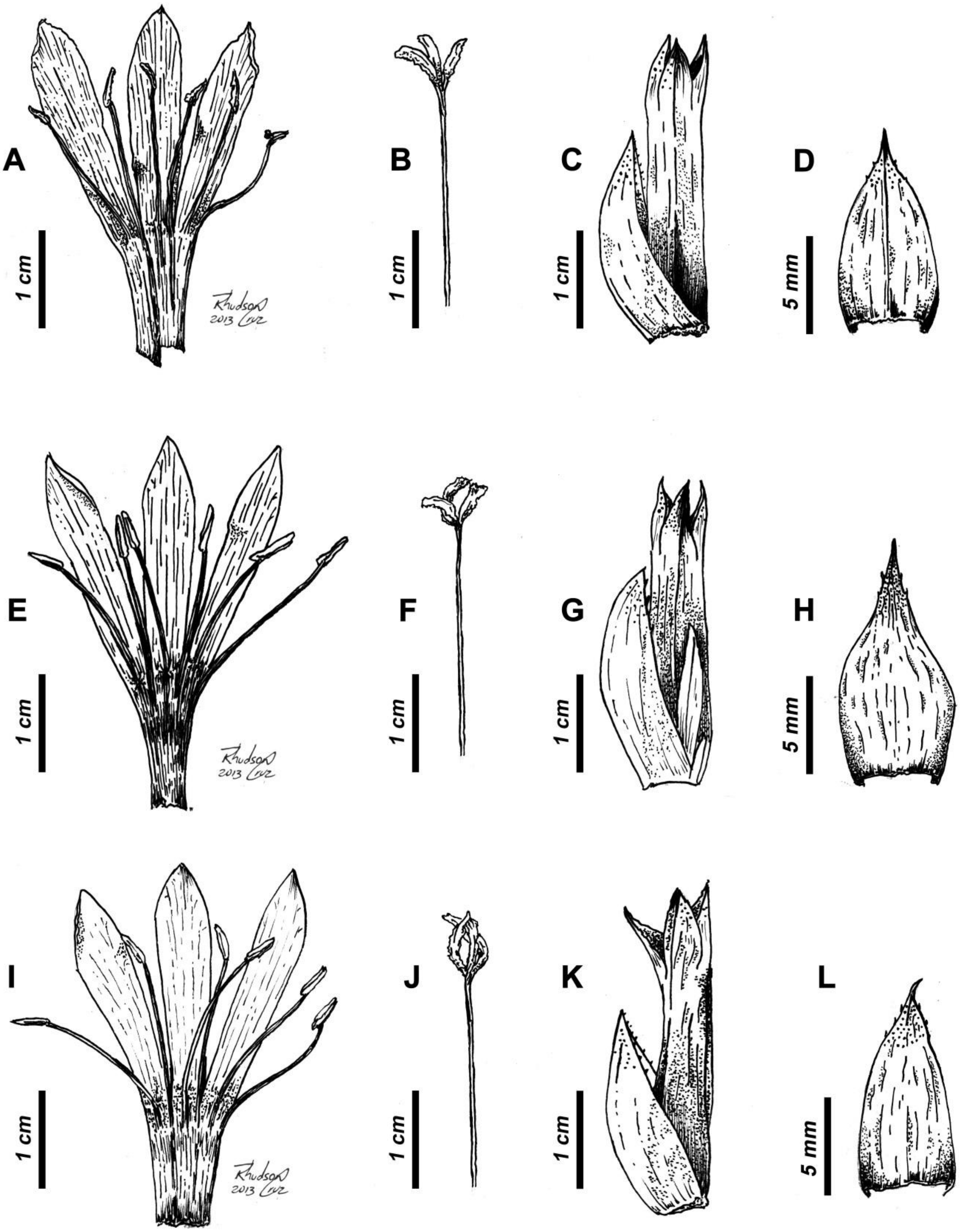
Figure 1: Map showing the studied areas (black triangles): the northern triangle represents the Parque Estadual das Dunas de Natal, the southern represents the RPPN Mata Estrela, Baía Formosa.

Source: DIVA, Own made

Figure 2: A: Bee (Apidae) on the anther of *C. zonatus* f. ***viridis*. B: Apidae bee on the anther *C. zonatus* f. *fuscus***

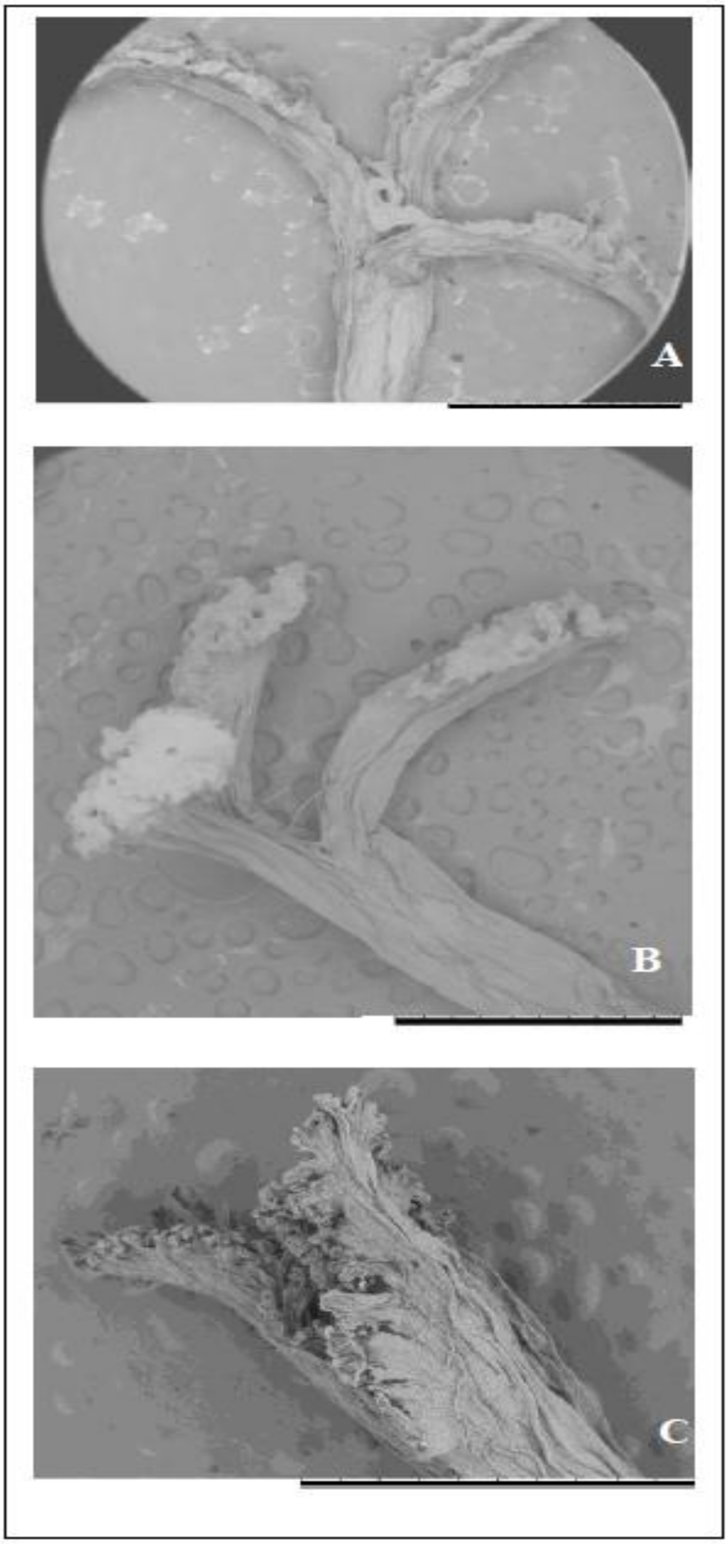
Source: Digital camera, Own made

Figure 3. ***Cryptanthus zonatus*** **floral morphology details for the three forms: From left to right (for all the drawings): Corolla and epipetalous stamens. Style and stigma. Floral bract and calyx. Floral bract front view. A-D.** ***C. zonatus*** **f. viridis. E-H.** ***C. zonatus*** **f.** ***fuscus*****. I-L.** ***C. zonatus*** **f.** ***zonatus*****.**



Source: handmade by Rhudson Henrique Santos Ferreira da Cruz

Figure 4: Stigma of the three forms of *Cryptanthus zonatus*. A. *C. zonatus* f. *viridis* B. *C. zonatus* f. *zonatus* and C. *C. zonatus* f. *fuscus*. Bar measures 2mm.



Source: Electronic microscopic Hitachi TM 3000

7. CONSIDERAÇÕES FINAIS

Com os resultados desse trabalho pode-se concluir que:

- Foi expandida a distribuição geral do gênero *Cryptanthus*, que agora inclui também o estado do Rio Grande do Norte;
- A espécie de *Cryptanthus* ocorrente no Rio Grande do Norte inclui-se na circunscrição de *C. zonatus*;
- A espécie *C. zonatus* é polimórfica com três morfos de cor, as quais ocorrem simpatricamente ou em populações separadas por pequenas distâncias;
- *C. zonatus* está incluída no livro vermelho de espécies ameaçadas de extinção no Brasil, entretanto, neste trabalho estabelece-se que a espécie encontra-se dentro de unidades de conservação no estado do RN: Parque estadual das Dunas e Reserva da Mata Estrela;
- Estudos demográficos mais detalhados devem ser realizados, mas estudos preliminares demonstram que a forma *zonatus* é mais rara e pode estar sofrendo com diminuição populacional e extrativismo ornamental;
- Estudos anatômicos indicam que os três morfotipos de *C. zonatus* não apresentam diferenças significativas na anatomia foliar com potencial de utilização na taxonomia do grupo;
- *Orthophytum disjunctum* apesar de apresentar padrão anatômico foliar bastante similar às formas de *C. zonatus*, apresenta diferenças significativas que são úteis para distinguir ambas as espécies;
- Há sobreposição do período de floração das formas de *C. zonatus* e pelo menos duas formas partilham visitantes florais;
- Há variação na morfologia floral em indivíduos de uma mesma população de *C. zonatus*, indicando que deve haver cautela na descrição de espécies novas baseadas unicamente em medidas das peças florais;
- A microscopia eletrônica de varredura demonstrou pouca diferença entre as formas de *C. zonatus*;

Abordagens futuras sugeridas incluem:

- Um estudo fenológico mais longo e detalhado pode ser realizado com as formas de C. zonatus para indicar mais seguramente os padrões de floração;
- Estudos fenológicos futuros devem incluir visitas mais frequentes dada a duração curta das flores de *C. zonatus*, e um maior número de flores deve ser marcada e observada;
- Análise reprodutiva das morfos de cor com cruzamentos controlados e a pesquisas de desenvolvimento de frutos e viabilidade de sementes;
- Estudos genéticos focados na compreensão da variabilidade genética do complexo *C. zonatus*.

8. APÊNDICE

Notes on geographic distribution

[4]*Aechmea muricata* (Arruda) L.B. Sm. (Bromeliaceae: Bromelioideae): a new record of a threatened species for Rio Grande do Norte, Northeastern Brazil

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Abstract

This work records the first occurrence of *Aechmea muricata* (Bromeliaceae, Bromelioideae) in the state of Rio Grande do Norte, northeastern Brazil. The taxon was found inside the Mata Estrela Private Reserve, Baía Formosa municipality, southern Rio Grande do Norte. This finding is important to increase the data about the Flora of Rio Grande do Norte, one of the poorest sampled states in Brazil so far, as well as to increase the knowledge about *A. muricata* distribution, since it is officially included in the Brazilian threatened species plant red list.

The Brazilian Atlantic rain forest retains today only 11.73% of its original area (RIBEIRO et al. 2009). It is considered to be a biodiversity hotspot due to the uniqueness of its biota, which is highly endangered mostly because of habitat loss (MYERS et al. 2000; RIBEIRO et al. 2009). Such forest is extremely important for Bromeliaceae due to high endemism, particularly for the Bromelioideae subfamily species living in this habitat (SMITH 1934). So far, only four genera and eight species of bromeliads were recorded in the Atlantic forest of Rio Grande do Norte (RN) (MARTINELLI et al. 2008). However, this data could be underestimated as a result of poor sampling for this family in RN. Rio Grande do Norte is considered one of the poorest Brazilian states in terms of floristic sampling and recent field surveys registered several new occurrences (VERSIEUX; TOMAZ; JARDIM 2013a; VERSIEUX; MAGALHÃES; CALVENTE 2013b). Therefore, any number presented for the Flora of Rio Grande do Norte not based in intensive and careful fieldwork investigation is probably outdated.

The eastern Brazilian rain forest is the center of diversity of *Aechmea* Ruiz & Pav., which comprises approximated 200 species and eight subgenera (SMITH; DOWNS 1979; LUTHER 2010). This genus is cited in the literature as the largest and is among the most taxonomically complex in the entire family (SMITH; DOWNS 1979). *Aechmea muricata* (Arruda) L.B. Sm. belongs to subgenus Chevaliera, which holds 21 species that are characterized by the presence of a strobiliform or capituliform and usually simple inflorescence, conspicuous peduncle bracts, flowers polystichously arranged and protected by a coriaceous floral bract (SMITH; Downs 1979, SOUSA; WANDERLEY 2000; CANELA ; LOPEZ PAZ; WENDT 2003, SILVA 2003; SOUSA et al. 2008). *Aechmea muricata* is remarkable by its cylindrical and green spicate inflorescence, by the peduncle covered by conspicuous bracts with entire margins and sharply pointed apex, as well as by its dark blue flowers (SMITH; DOWNS 1979, SOUSA; WANDERLEY 2000; SOUSA; WANDERLEY; ALVES 2008). This species is restricted to the northeastern Brazil, occurring at sandy areas near the seacoast, being either a terrestrial or an epiphyte (SMITH; DOWNS 1979; SOUSA; WANDERLEY 2000; LEME; SIQUEIRA-FILHO 2006). Currently, this taxon has been cited only for Pernambuco and Alagoas States (FORZZA et al. 2013) and it is officially included in the Brazilian threatened plant species red list (MMA 2008). Here we document the first vouchered occurrence of *A. muricata* for the state of Rio Grande do Norte.

The Mata Estrela Private Reserve is located at the municipality of Baía Formosa (06º22’10”S 35º00’28”W), close to the border of Rio Grande do Norte and Paraíba states (Fig. 1). This new record for *A. muricata* represents an increase in approximately 180 km toward north in the distributional range of this species. Only one small population of *A. muricata* was observed in Mata Estrela. There, the individuals present a terrestrial habit, occurring in sandy soil and sunny open habitat, in the coastal sand plain scrub vegetation. The specimen was photographed in the field, collected and deposited in the herbarium of the Federal University of Rio Grande do Norte (UFRN; R. Magalhães 19) (Fig. 2). *Aechmea muricata* is currently on the list of Brazilian endangered species (MMA 2008) and this new record is particularly important for setting strategies for its conservation and to document its occurrence inside a reserve. This new record is also important to increase the information and to update the data about the Flora of Rio Grande do Norte, which still remains poorly known (VERSIEUX; TOMAZ; JARDIM 2013a). Also in Mata Estrela, another new occurrence was recently registered for the genus Cryptanthus (Bromeliaceae, Bromelioideae) (VERSIEUX; MAGALHÃES; CALVENTE 2013b), what points to the need of further investigating this poorly known area and the adjacent remnants of the Rio Grande do Norte Atlantic Rain Forest.

`Figura 1:` **Map of Northeastern Brazil, showing in pale yellow the states where *Aechmea muricata* occurs. The new occurrence in Rio Grande do Norte is indicated by a red triangle, expanding in ca. of 180 km toward north from earlier records (green triangles).**

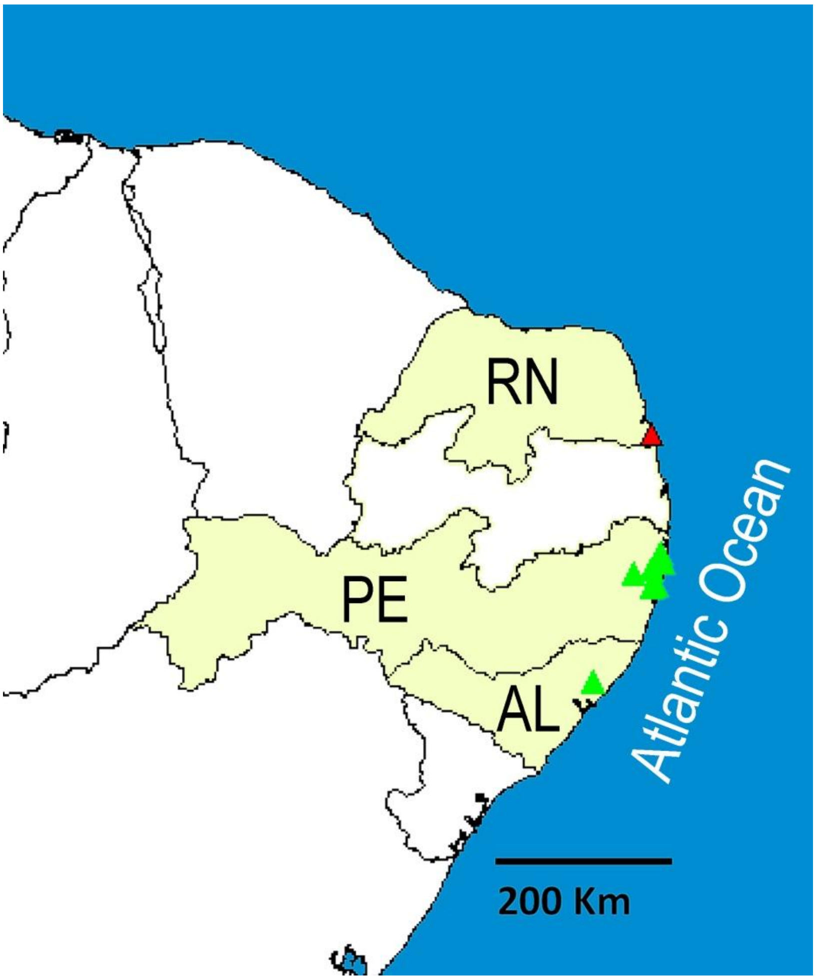

Figura 2: A, B: Specimen of *Aechmea muricata* collected in Baía Formosa, Rio Grande do Norte (Voucher: Magalhães 19, UFRN). C: photo of a living plant in the field. Photos: R. Magalhães

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