Die Rolle von Bienen und anderen Blütenbesuchern in der Reproduktionsbiologie der Bromelien *Aechmea nudicaulis* und *Vriesea friburgensis* im Atlantischen Regenwald Südbrasiliens

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„Plants are not pollinated
by latin binominals.“

Jeff Ollerton

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1 Einleitung

Im Rahmen gemeinsamer Umweltschutzvorhaben wurde 2001 das deutsch-brasilianische Kooperations-Programm „Mata Atlantica“ eingerichtet. Es umfasst eine Reihe von regionalen Forschungsvorhaben, darunter im Bundesstaat Santa Catarina das Projekt „Interne Dynamik des Regenwaldes: spezifische Tier-Pflanze-Interaktionen“, geleitet von Dr. Anne Zillikens (Universität Tübingen) und Prof. Dr. Josefina Steiner (Federale Universität von Santa Catarina, Florianópolis). Innerhalb dieses Projektes bildeten Untersuchungen zu Wechselbeziehungen zwischen Bromelien und Tieren einen Schwerpunkt.

Mein Promotionsthema hat eine Reihe von reproduktionsbiologischen Fragen zum Inhalt, die exemplarisch an zwei Bromelienarten analysiert wurden. Neben Tier-Blüte-Interaktionen im Allgemeinen wurden die Bestäubungsbiologie und speziell die Effektivität verschiedener Blütenbesucher als Bestäuber untersucht.

Vom Atlantischen Küstenregenwald Brasiliens, welcher ehemals 15% Ostbrasiliens bedeckte, sind heute nur noch etwa 7,5% der ursprünglichen Fläche übrig (Scarano 2002). Aufgrund der Artenvielfalt und des hohen Anteils an endemischen Arten, sowie der Bedrohung dieses Lebensraumes, wird der Atlantische Regenwald als „Hotspot“ der Biodiversität angesehen (Myers et al. 2000; UNESCO Natural Sciences 2007). Um die hohe Artenvielfalt zu erhalten, ist es notwendig, die biologischen Zusammenhänge zu erforschen und die Tier-Pflanze-Interaktionen zu untersuchen. Eine allgegenwärtige Art von Interaktion ist die Bestäubung vieler Pflanzenarten durch Blütenbesucher. Die Übertragung der Pollenkörner von männlichen auf weibliche Blütenteile ist für die meisten Vertreter der Samenpflanzen (Spermatophytae) die Voraussetzung für Befruchtung und Samenbildung. Anders als in den gemäßigten Zonen findet sich in den Regenwäldern der Tropen und Subtropen nur selten Windbestäubung (Anemogamie, Whitmore 1993), da die Individuen einer Art oft sehr weit auseinander stehen. Die meisten Pflanzenarten sind deshalb auf die Pollenverbreitung durch Tiere angewiesen (Zoogamie). Man unterscheidet bei der Bestäubung unterschiedliche Syndrome. Mit dem Begriff Bestäubungssyndrom werden Gruppen von Blütenmerkmalen (phänologische Merkmale wie Blütengröße, -form, oder -farbe) als auch die Belohnungen (Nektar und Pollen) zusammengefasst und einer bestimmten Gruppe von Blütenbesuchern – zum Beispiel Vögeln, Fledermäusen oder Bienen – zugeordnet. Diese werden durch die attraktiven Merkmale zu den Blüten gelockt und bestäuben sie (Fenster et al. 2004). Vogelbestäubte Blüten (ornithophiles Syndrom) zeichnen sich meist durch röhrenförmige und stabile Blüten in auffallenden Farben (rot, orange, gelb und grün) aus. Die Blüten sind tagsüber geöffnet und verströmen in der Regel keinen Duft. Charakteristisch ist Nektar mit niedrigem Zuckergehalt und einem hohen Saccharose-Anteil. Bienenblüten (melittophiles Syndrom) sind oftmals zygomorph und auffällig gefärbt und verfügen über eine Landeplattform und Saftmale, welche die Bienen zum Nektar führen. (Faegri & van der Pijl 1971; Whitmore 1993).

Neuere Studien haben jedoch gezeigt, dass auch Besucher, die nicht zum Blütensyndrom passen, als Bestäuber fungieren können (Waser et al. 1996; Johnson & Steiner 2000; Mayfield et al. 2001; Frank & Lounibos 2008; Ollerton et al. 2009). Ein Beispiel dafür ist die Bestäubung der Blüten einer ornithophilen Mistel durch Bienen (Robertson et al. 2005). In diesem Fall werden die Bienen als sekundäre oder alternative Bestäuber angesehen. Für einige Pflanzen wurden auch sogenannte bimodale Bestäubungssysteme nachgewiesen. In einem solchen System können die Blüten nicht einem einzigen Syndrom zugeordnet werden, sondern zeigen Anpassungen an zwei unterschiedliche Bestäubergruppen (zum Beispiel Vögel und Bienen) und werden von Vertretern dieser Gruppen bestäubt (Manning & Goldblatt 2005; siehe auch Waser & Price 1990; Castellanos et al. 2003; Shuttleworth & Johnson 2008). Bei zwei Arten von Iridaceae (*Tritoniopsis toximontana* and *T. pulchella*) wurden die Blüten von Bienen und langzüngigen Fliegen bestäubt. Die Blütenlänge erlaubte Besucher beider Gruppen und die Zuckerkonzentration war für Bienen und Fliegen gleichermaßen attraktiv. Bei *T. burchellii* und *T. triticea* wurde die Bestäubung durch Nektarvögel und Schmetterlinge nachgewiesen (Manning & Goldblatt 2005).

Die neotropischen Bromelien (Bromeliaceae) sind eine Pflanzenfamilie mit etwa 3000 Arten (in acht Unterfamilien; Givnish et al. 2007) aus der monokotylen Ordnung der Süßgrasgewächse (Poales). Vertreter dieser Familie sind im allgemeinen krautige Gewächse, die sowohl epiphytisch als auch auf dem Boden oder auf Felsen wachsen. Die Wuchsform der meisten Bromelienarten ist eine Blattrosette. Bromelien, deren Rosette Zisternen (“Phytotelmata“) bilden, in denen sich Wasser und Humus ansammeln, nennt man Trichterbromelien (“tank bromeliads“, Frank & Lounibos 2008). Das in der Rosette gespeicherte Wasser bzw. der dort akkumulierte Humus sind Mikrohabitate und Nahrungsgrundlage für eine Vielzahl aquatischer und terrestrischer Tierarten (Frank & Lounibos 2008). Die enge Verknüpfung bestimmter Tiergruppen (z.B. aquatische Invertebraten oder speziell Moskitos) oder die Vielfalt aller assoziierten Tiere mit Bromelien waren Gegenstand vieler Untersuchungen (Müller 1879; Picado 1913; Richardson 1999; Wittman 2000; Mestre et al. 2001; Frank et al. 2004; Müller & Marcondes 2007; Frank & Lounibos 2008; Schmid et al. 2010b). Aber nicht nur die Bewohner der Rosette, z.B. Ameisen als Beschützer der von ihnen bewohnten Pflanze (Vesprini et al. 2003), oder Spinnen, deren Ausscheidungen als Dünger genutzt werden (Romero et al. 2006), sondern vor allem auch die Blütenbesucher und Samenfresser sind ein für die Bromelien essentieller Faktor ihres Lebenszyklus (Frank & Lounibos 2008). Ihre Blütenstände tragen in erheblichem Maße zum Erhalt der Biodiversität der lokalen Fauna bei, indem sie einem breiten Spektrum von Vögeln und Arthopoden Nahrung bieten (Sazima & Sazima 1999; Machado & Semir 2006; Canela & Sazima 2003; Schmid et al. 2010a).

Viele Bromelien haben augenfällige Blütenstände (Benzing 2000), deren Blüten überwiegend durch Tiere (Zoogamie) bestäubt werden. Selbstbestäubung (Autogamie) kommt dagegen selten vor (Benzing 2000). Unter den zoogamen Arten dominiert die Bestäubung durch Wirbeltiere wie Kolibris oder Fledermäuse über die Bestäubung durch Insekten (Sazima et al. 1989), wobei man Kolibribestäubung am häufigsten beobachtet (Benzing 2000; Kessler & Krömer 2000). Bromelien sind im Atlantischen Regenwald eine bedeutende Nektarquelle für Kolibris (Buzato et al. 2000; Cestari 2009). Ob Blütenbesucher effektive Bestäuber oder neutrale Besucher sind, oder ob sie sogar einen negativen Einfluss auf die Bestäubung haben, hängt nicht nur davon ab, zu welcher Gruppe (Vögel, Bienen, Schmetterlinge) sie gehören, sondern vor allem, wie sie sich an den Blüten verhalten (Théry et al. 1998). Besucher, welche die reproduktiven Organe der Blüten berühren, können als Bestäuber fungieren, dürfen aber nicht automatisch als solche betrachtet werden. Das Bestäubungssystem der Blüten, die Morphologie und das Sammelverhalten der Blütenbesucher müssen bei der Beurteilung in Betracht gezogen werden. Besucher, die die angebotene Nahrung sammeln, ohne einen Kontakt zu Antheren und Stigma herzustellen, oder sogar die Blüten dabei zerstören, haben einen negativen Einfluss auf die Reproduktion. Eine Beschädigung der Blüten kann die Ausbildung von Samen verhindern. Nektarraub macht die Blüten für die eigentlichen Bestäuber weniger attraktiv, was dazu führen kann, dass diese die Blüten seltener besuchen. Die Ausbeutung des Pollens (insbesondere durch Bienen) entfernt diesen aus dem Reproduktions-Kreislauf, so dass er nicht mehr für die Bestäubung anderer Blüten zur Verfügung steht. Die Konsequenz ist ein verringerter Bestäubungserfolg. Nektar ist die Energiequelle für die Blütenbesucher (Krömer et al. 2008), da Pollen hauptsächlich als Proteinquelle dient. Kolibris sind darauf spezialisiert, Nektar aus Blüten zu trinken, so dass die Qualität und das Volumen des Nektars in den Blüten zwei Auswahlkriterien für diese Vögel sind. In einigen Studien wurden für Bromelien Daten zu Nektarvolumen und Zuckerkonzentrationen erhoben. Krömer et al. (2008) lieferten dabei einen umfassenden Bericht über die Nektarkonzentration und -zusammensetzung von 111

Bromelienarten. Bernardello et al. (1991) untersuchten die chemische Nektarzusammensetzung von 20 Bromelienarten, setzten sie aber nicht mit dem Volumen in Beziehung. Canela & Sazima (2003) untersuchten Nektarvolumen und Konzentration in 2-stündigen Intervallen für *Ae. pectinata*. Dabei fanden sie heraus, dass die Nektarproduktion und auch die Zuckerkonzentration morgens am höchsten waren. Dieses tageszeitliche Muster in der Nektarproduktion war in ihrer Studie positiv mit der Häufigkeit der Kolibribesuche korreliert. Laut Krömer et al. (2008) beinhaltet Bromeliennektar ausschließlich Saccharose und die beiden Hexosen Glukose und Fruktose. Das Verhältnis von Saccharose zu den Hexosen scheint dabei einen Hinweis auf die möglichen Bestäuber zu geben. Obwohl das tageszeitliche Muster der Zuckerzusammensetzung und des Volumens des Nektars einen starken Einfluss auf die Attraktivität der Blüten für die Blütenbesucher hat, wurde dieser Zusammenhang an Bromelienblüten bisher nicht untersucht.

Auf der Insel von Santa Catarina kommt in den Habitaten Sekundärwald und Restinga (s.u.) eine große Vielfalt an Bromelienarten vor. Sie unterscheiden sich beträchtlich in Farbe (von weiß über gelb, orange und rot bis zu rosa und violett) und Form der Blüten (von offen bis stark röhrenförmig) als auch der Struktur der Infloreszenzen (Blüten tief unten in der Rosette knapp oberhalb des Wasserspiegels oder an hohen, z.T. verzweigten Blütenstand) und ihrer Blühperiode und Saisonalität. Es ist daher offensichtlich, dass die Blüten mehreren Blütensyndromen zuzuordnen sind. Dies ermöglicht es, das Spektrum der erwarteten Blütenbesucher bzw. Bestäuber mit dem der tatsächlich beobachteten zu vergleichen und so die Aussagekraft von Blütensyndromen zu überprüfen.

Für meine Untersuchungen habe ich die beiden Bromelienarten *Aechmea nudicaulis* (Bromelioideae) und *Vriesea friburgensis* (Tillandsioideae) als Modellorganismen ausgewählt. Sie überschneiden sich nur wenig in ihrer Blühperiode, sie sind aber sowohl in Sekundärwäldern als auch in Dünengebieten gemeinsam (syntopisch) anzutreffen. Daher sind sie in den jeweiligen Habitattypen derselben Bestäubergemeinde ausgesetzt. Beide Bromelienarten haben Blüten mit ornithophilen Merkmalen. Diese sind bei *Vriesea* allerdings stärker ausgeprägt: die Blüten haben längere Kronröhren als *Aechmea* und, anders als diese, exponierte Antheren und Stigmen.

Über das Fortpflanzungssystem der beiden Bromelienarten sind nur wenige, teils anekdotische Fakten bekannt. Tatsächlich wurden Kolibris als Blütenbesucher sowohl an *Ae. nudicaulis* im Atlantischen Regenwald im Staat São Paulo (Buzato et al. 2000; Machado & Semir 2006), als auch an *V. friburgensis* im Litoral von Paraná beobachtet (Piacentini &

Varassin 2007). Zusätzlich gibt es einige Berichte von Insekten, zum Beispiel Bienen und Schmetterlingen, als Besucher von vogelblütigen Bromelien (Bernardello et al. 1991; Wendt et al. 2001, 2002; Canela & Sazima 2003). Insbesondere kurzröhrige Bromelien, die aber ansonsten ein ornithophiles Syndrom zeigen, werden von Bienen besucht (Canela & Sazima 2003). Bei der Untersuchung von Nestern der Prachtbiene *Euglossa annectans* (Euglossinae), konnte *Aechmea-* und *Vriesea*-Pollen in den Brutzellen nachgewiesen werden (Cortopassi-Laurino et al. 2009). Solche Beobachtungen können ein Hinweis auf bimodale Bestäubungssysteme sein. Nur durch genaue Feldbeobachtungen und Experimente kann man eindeutig bestimmen, welche Besucher als Bestäuber und welche nur als Ressourcenräuber tätig sind (e.g. Keys et al. 1995; Freitas & Paxton 1998; Botes et al. 2009; Bumrungsri et al. 2009), doch bis jetzt wurden keine solchen Tests zur Bestäubungseffektivität vogelblütiger Bromelien durchgeführt. Da die Prachtbienen offensichtlich vom Nahrungsangebot der Blüten von *Ae. nudicaulis* und *V. friburgensis* profitieren, stellte sich die Frage, ob sie auch als Bestäuber fungieren können, oder, allgemeiner formuliert, ob blütenbesuchende Insekten bei der Bestäubung dieser Bromelien eine Rolle spielen.

Beobachtungen im Feld ergaben, dass viele Blüten von *V. friburgensis* nicht zu Früchten reiften. Untersuchungen solcher unentwickelter Früchte im Labor zeigten, dass die Knospen von Wespen der Gattung *Eurytoma* (Eurytomidae) befallen waren. Ein Großteil der Arten dieser Familie sind endophytische und phytophage Pflanzenparasiten oder Parasiten von Pflanzenschädlingen (Noyes 2003). Zu den Pflanzenparasiten gehört *Eurytoma werauhia*, die die Knospen der Bromelie *Werauhia gladioliflora* befällt (Gates & Cascante-Marín 2004). Ein Beispiel für einen Parasiten ist *Eurytoma aerflora*, deren Wirt eine Bromelienfrüchte fressende Schmetterlingsraupe ist (Bugbee 1975). Schmetterlinge stellen eine große Gruppe von herbivoren Schädlingen an Bromelien. Ein Beispiel dafür ist die Art *Napaea eucharilla* Bates (Riodinidae), deren Raupen an Früchten der Arten *Werauhia sanguinolenta*, *Ae. bracteata*, *Ae. nudicaulis* und *Ananas comosus* (kommerziell angebaute Ananas) fressen. Auch Bläulinge der Gattung *Strymon* (Neuweltliche Zipfelfalter, Lycaenidae: Theclinae: Eumaeini) sind bekannte Schädlinge an Zierbromelien und Fruchtschädlinge von Ananaspflanzen (*An. comosus*) (Robbins & Nicolay 2002). Die Art *Strymon megarus* wird an *An. comosus* und *Ae. bracteata* gefunden (Beutelspacher 1972; Frank & Lounibos 2008). Aktuell sind nur für etwa 25% der Eumaeini die Wirtspflanze bekannt, da die Eier, Raupen und Puppen klein und unauffällig oder sogar in ihrer Färbung an die Wirtspflanzen angepasst (kryptisch) sind und somit nur schwer entdeckt werden (Duarte et al. 2005).

In der Familie der Bläulinge sind viele Arten mit Ameisen assoziiert (Pierce et al. 2002). Das Spektrum der Interaktionen reicht von einfachen Beziehungen, in denen die Ameisen mit Ausscheidung nektarähnlicher Sekrete besänftigt werden (Malicky 1970), bis hin zu komplexen spezifischen Assoziationen, in denen die Raupen ins Ameisennest eingetragen werden und sich dort von der Ameisenbrut ernähren (Grupp 2009). Bisher sind keine Interaktionen von Bläulingen und den in meiner Studie untersuchten Bromelien bekannt.

Die hohe Abundanz und die Vorherrschaft von Ameisen in fast allen terrestrischen Habitaten (Hölldobler & Wilson 1990; Alonso & Agosti 2000) macht sie zu einem wesentlichen Bestandteil der Fauna vieler Ökosysteme. Beziehungen zu Pflanzen sind häufig anzutreffen (Hölldobler & Wilson 1990; Huxley 1991). Diese sind, ähnlich wie die zwischen Bläulingen und Ameisen, variabel in ihrer Ausprägung von losen bis hin zu hochentwickelten spezifischen Assoziationen, zum Beispiel mit Akazien (Wheeler 1912; Janzen 1966; Heil et al. 2005) oder *Cecropia* (Janzen 1969; Schupp 1986). Auch Bromelien sind auf vielfältige Weise mit Ameisen assoziiert (Frank & Lounibos 2008). Ein Beispiel dafür sind in Bromelienpflanzen angelegte Ameisennester (Blüthgen et al. 2000; Cogni & Oliveira 2004; Rosumek et al. 2008). Möglicherweise gehen Ameisen mit Bromelien mutualistische Beziehungen ein, in denen die Blüten extrafloralen Nektar für die Ameisen produzieren und diese im Gegenzug Herbivore verjagen (Vesprini et al. 2003). An zwei in unseren Untersuchungsgebieten auf der Insel von Santa Catarina vorkommenden Bromelienarten, *Ae. lindenii* und *Ae. nudicaulis*, konnten oft Ameisen an den Infloreszenzen beobachtet werden. Dabei war nicht offensichtlich, ob die Ameisen dort extrafloralen Nektar aufnahmen (morphologische Strukturen, die auf eine Nektarsekretion hinweisen, waren mit bloßem Auge nicht erkennbar) oder Jagd auf die häufig vorhandenen Blütenmilben machten. Diese werden von Kolibris zwischen den von ihnen besuchten Blüten transportiert. Solche Fälle von Phoresie sind auch von anderen Systemen bekannt (Colwell et al. 1974; Lara & Ornelas 2002). Blütenmilben ernähren sich von floralem Nektar (Colwell 1973, 1995; Colwell & Naeem 1994; da Cruz et al. 2007) und Pollen (Colwell & Naeem 1994; Paciorek et al. 1995) und haben somit wahrscheinlich einen gewissen Einfluss auf die Reproduktion der Bromelien.

Zielsetzungen

1) Analyse der Bestäubungssysteme und der Diversität der Blütenbesucher

- Die reproduktiven Systeme beider Bromelienarten wurden analysiert (Veröffentlichung 4 und 6). Sind die beiden Bromelienarten autogam oder xenogam? Entsprechen die Blütenmerkmale einem Syndrom oder sind Übergänge zu anderen Syndromen erkennbar? Neben einer Analyse der Blütenmorphologie und der Blühphasen wurden Nektaruntersuchungen durchgeführt, da anhand des Nektars auch Rückschlüsse auf die gewünschten Bestäuber gezogen werden können.
- Wie effizient bestäuben die häufigsten Blütenbesucher (Veröffentlichung 4)? Sind alle Blütenbesucher Bestäuber? Passen die experimentell ermittelten Bestäuber zum jeweiligen Blütensyndrom?
- Die Diversität der Blütenbesucher im weitesten Sinne wurde erhoben (Veröffentlichung 5 und 6). Welche Besuchsfrequenzen haben die einzelnen Taxa: gibt es eine zeitliche Einnischung? Welcher Besucher sammelt welche Ressourcen und wie werden diese gesammelt? Inwiefern hat die Blütenmorphologie darauf einen Einfluss? Da Bienen ein morphologisch hochdiverses Taxon sind, sollte analysiert werden, welche Unterschiede es in ihrer Eignung als Bestäuber gibt. Dazu wurden Verhaltensbeobachtungen an den Infloreszenzen durchgeführt.
- Welche Interaktionen gibt es zwischen den Blütenbesuchern (Veröffentlichung 5 und 6)?

2) Welche Blütenbesucher im weitesten Sinne haben neben den Bestäubern unmittelbar vor, während, oder nach der Bestäubung einen Einfluss auf den Reproduktionserfolg der Bromelien?

- Welche Organismen sind die Ursache für die ausbleibende Entwicklung einiger Blütenknospen von *V. friburgensis* (Veröffentlichung 1)? Wie erfolgt die Sterilisierung der Blüten? Wie spezifisch ist die Beziehung der Schädlinge zu den Bromelien, und wie verläuft ihr Lebenszyklus?

- Welche Schädlinge greifen Früchte von *Ae. nudicaulis*, *V. friburgensis* und weiteren *Aechmea*-Arten an (Veröffentlichung 2)? Wie viele Schädlingsarten sind mit diesen Bromelienarten auf der Insel von Santa Catarina assoziiert, und wie wirtsspezifisch sind sie? Wie verläuft der Lebenszyklus der Schädlinge? Welche ihrer Entwicklungsstadien sind wie mit den Bromelien assoziiert?

- Aufnahme der Diversität der assoziierten Ameisen (Veröffentlichung 3). Was ist der Grund für ihre Anwesenheit an den Blütenständen – das Sammeln von extrafloralem Nektar oder die Jagd auf Blütenmilben?
- Welche Rolle spielen die Blütenmilben für die Bromelienreproduktion (Veröffentlichung 3)? Ernähren sie sich von Pollen?

3) Einfluss von Habitat und Breitengrad

- Wie weit beeinflussen unterschiedliche Habitattypen wie Sekundärwald und Restinga beziehungsweise Unterschiede im Breitengrad (Santa Catarina vs. Paraná) die Blütenbesucherspektren von *Ae. nudicaulis* und *V. friburgensis* (Veröffentlichung 4 und 6)?

2 Material & Methoden

2.1 Die Testorganismen: *Aechmea nudicaulis* und *Vriesea friburgensis*

Verbreitungs- und Untersuchungsgebiete

Aechmea nudicaulis (L.) Grisebach (Bromeliaceae: Bromelioideae) hat ein weites Verbreitungsgebiet von Südbrasilien bis Mexiko und kann in den Küstenregionen im brasilianischen Bundesstaat Santa Catarina (SC) häufig angetroffen werden (Reitz 1983). Diese Art kommt sowohl in natürlicher (Sekundärwald und Dünengebiete) als auch in städtischer Umgebung (in Gärten und Parks) vor. *Vriesea friburgensis* ist endemisch in Brasilien und bevorzugt in SC hellere Standorte wie die Dünengebiete und lichte, noch junge Wälder. In älteren und dichteren Wäldern wurde sie nicht gefunden.

Die Feldstudien zu meiner Arbeit wurden im Atlantischen Regenwald im Süden Brasiliens durchgeführt. Die Untersuchungsgebiete lagen auf der Insel von Santa Catarina, im Stadtbezirk Florianópolis (SC), und in einem Naturschutzgebiet im Litoral des Bundesstaates Paraná (PR). In SC wurden folgende Gebiete ausgewählt: (i) zwei ineinander übergehende Dünengebiete an den Stränden von Joaquina und Campeche mit niedriger Strauch- und Krautvegetation auf Sandboden, so genannte ‘Restinga’ (Sampaio et al. 2002); (ii) RPPN Morro das Aranhas (Private Reserve of Natural Heritage), ebenfalls ein Restinga-Gebiet, jedoch mit dichterem und höherem Bewuchs als in Joaquina and Campeche; und (iii) zwei Gebiete eines zusammenhängenden Sekundärwaldes mit Miconietum (ein frühes Sukzessionsstadium des Sekundärwaldes, in welchem kleine Bäume der Gattung *Miconia* dominieren) an den Rändern. Das erste liegt an einem Berghang bei der Ortschaft Santo Antônio de Lisboa (ab hier “Santo Antônio“ abgekürzt; Zillikens et al. 2001; 27°30’26’’ S, 48°30’28’’ W), das zweite in einem Naturschutzgebiet (UCAD; 27°31′50″S, 48°30′50″W) im Besitz der Föderalen Universität von Santa Catarina (UFSC) mit etwa 500 ha Sekundärwald und Miconietum (Zillikens & Steiner 2004). Im Schutzgebiet in PR findet sich auf ehemaligen Weidegebieten nun Sekundärwald in unterschiedlichen Sukzessionsstadien. Die Untersuchungen wurden in einem schon fortgeschrittenen Sukzessionsstadium (Baumhöhe 15-20 m) durchgeführt.

Lebensweise und Morphologie

Pflanzen der Art *Ae. nudicaulis* wachsen auf der Insel von SC hauptsächlich am Boden oder auf Felsen, Individuen von *V. friburgensis* wachsen nur auf dem Boden. Die Rosetten werden von breiten, zungenförmigen Blättern gebildet, die im Fall von *Ae. nudicaulis* deutlich gezähnt sind. Beide Bromelien vermehren sich neben der sexuellen Fortpflanzung auch ungeschlechtlich durch Kindelbildung. Die Ableger von *Ae. nudicaulis* sind über ein Rhizom mit der Mutterpflanze verbunden und bilden häufig flächige Gruppen von genetisch identischen Pflanzen. Bei *V. friburgensis* hingegen bilden sich die Kindel innerhalb der Rosette zwischen den Blattachseln der Mutterpflanze. Die Klone haben somit keine flächige Verbreitung sondern bilden engstehende Ansammlungen von einzelnen Rosetten (Sampaio et al. 2002). Beide Arten sind monokarp, d.h. aus jeder Rosette wächst nur einmal ein Blütenstand und nach der Fruchtreife stirbt die Rosette ab (Whitmore 1993). Die Infloreszenzen von *Ae. nudicaulis* sind sehr auffällig gefärbt und etwa 30-40 cm lang. Die Brakteen (Hochblätter) sind leuchtend rot und die Blüten sitzen direkt am Stiel (siehe Abb. 1A, Veröffentlichung 4). Der etwa 1 m lange, rötliche Blütenstand von *V. friburgensis* verzeigt sich am oberen Ende. Die Blüten sitzen an den Seitenästen und der Spitze des Haupttriebes (siehe Abb. 1, Veröffentlichung 1). Die gelben Blüten beider Bromelienarten sind radiärsymmetrisch, röhrenförmig und verströmen keinen für die menschliche Nase erkennbaren Duft. In den zwittrigen Blüten befinden sich sechs Antheren. Diese sind bei *Ae. nudicaulis* nicht exponiert und stehen kreisförmig angeordnet kurz hinter der Blütenöffnung. Das Stigma befindet sich in ihrer Mitte (siehe Abb. 1C in Veröffentlichung 4). Hingegen ragen die Antheren und das Stigma bei *V. friburgensis* weit aus der Blütenkrone heraus (siehe Abb. 9D, Veröffentlichung 6). Die Früchte von *Ae. nudicaulis* sind fleischige, rote Beeren, während *V. friburgensis* trockene Kapseln mit Flugsamen bildet.

Die detaillierten Untersuchungsmethoden zu den folgenden Abschnitten finden sich in den jeweiligen Veröffentlichungen.

2.2 Die parasitische Wespe *Eurytoma* sp. als Fruchtschädling von *Vriesea friburgensis* (Veröffentlichung 1)

Von Blütenständen der Bromelie *V. friburgensis* wurden im März 2005, Mitte September und Oktober 2006 in Sto Antônio und Joaquina Zweige mit nicht geöffneten Knospen, verwelkten Blüten und Früchten gesammelt und ins Labor gebracht. Dort wurden die Knospen aufgeschnitten und auf Wespen bzw. deren Larven hin untersucht.

2.3 Die Beziehung von Bromelien und Bläulingen (Lycaenidae) (Veröffentlichung 2)

Zwischen November 2006 und Juni 2008 wurden in vier Gebieten auf der Insel von Santa Catarina (Santo Antônio, UCAD, Joaquina und Campeche) Blütenstände von drei Bromelienarten auf den Befall durch Bläulingsraupen (charakteristischer Fruchtfraß, Larven und Eier) hin untersucht. Befallene Pflanzen wurden ins Labor überführt und die Entwicklung der Raupen wurde protokolliert.

2.4 Beziehungen von Ameisen und Bromelienblüten: Wodurch werden die Ameisen angelockt? (Veröffentlichung 3)

Um zu klären, in welcher Beziehung Ameisen und die von ihnen besuchten Blütenstände stehen, wurden von August 2005 bis Januar 2006 Feldstudien an den Bromelienarten *Ae. lindenii* und *Ae. nudicaulis* durchgeführt. Die Versuche erfolgten in fünf Untersuchungsgebieten auf der Insel Santa Catarina: UCAD, Santo Antônio, RPPN Morro das Aranhas, Joaquina und Campeche.

Blütenstände von *Ae. lindenii* und *Ae. nudicaulis* wurden in drei Blühphasen – „Knospen“, „offen“ und „verblüht“ – eingeteilt und die Blütenstände auf Ameisen und Blütenmilben hin untersucht. Um zu ermitteln, was die Blütenstände für Ameisen attraktiv macht, wurden das Verhalten und der dafür eingesetzte Zeitaufwand von 208 Ameisen (13 Arten) an offenen Blüten beobachtet. Der Einfluss von Milben auf die Pollenverfügbarkeit wurde untersucht. Um die Produktion von extrafloralem Nektar nachzuweisen, wurden bei Pflanzen im Labor die Blütenstände in Plastiktüten eingebeutelt und auf Nektarsekretion überprüft.

2.5 Die Blütenbiologie von *Aechmea nudicaulis* und *Vriesea friburgensis* (Veröffentlichungen 4 und 6)

Die Feldstudien zur Blütenbiologie von *Ae. nudicaulis* und *V. friburgensis* wurden im Atlantischen Regenwald im Süden Brasiliens durchgeführt. Blühende Pflanzen beider Bromelienarten wurden von September bis Dezember in den Jahren 2005 bis 2008 untersucht. Die Untersuchungsgebiete lagen auf der Insel von Santa Catarina, im Stadtbezirk Florianópolis. Es wurden drei Untersuchungsgebiete ausgewählt: (i) Joaquina und Campeche (krautige Restingavegetation); (ii) RPPN Morro das Aranhas (hochwüchsige Restingavegetation); und (iii) Santo Antônio (Sekundärwald).

Daten zur Blühperiode, der Gesamtzahl der Blüten und zum Vorgang der Blütenöffnung wurden erhoben. Das Bestäubungssystem der beiden Bromelienarten wurde in Handbestäubungsversuchen ermittelt. Um den natürlichen Bestäubungserfolg unter natürlichen Verhältnissen zu bestimmen, wurden bei sonnigem Wetter im Feld offene Blüten markiert und nach der Fruchtreife die Fruchtentwicklung und die Samenzahl bestimmt. Die Zeitspanne für eine mögliche Bestäubung durch Blütenbesucher wurde im Labor mit Hilfe von Kreuzbestäubungsversuchen untersucht. Versuche zur Nektarproduktion der beiden Bromelienarten wurden an blühenden Pflanzen im Labor durchgeführt. Die Zuckeranteile im Nektar wurden durch Hochdruck-Flüssigkeitschromatographie (HPLC) ermittelt (Piechowski 2007).

2.6 Die Bestäubereffizienz bei *Aechmea nudicaulis* (Veröffentlichung 4)

Um die Effizienz zu ermitteln, mit der die einzelnen Besuchergruppen die Bromelienblüten bestäuben, wurden in den Jahren 2006 und 2008 Bestäubungsexperimente in Santo Antônio und Campeche durchgeführt. Die Samenproduktion nach einem Besuch eines einzigen Individuums pro Blüte wurde ermittelt und in Beziehung zur Besuchsfrequenz des jeweiligen Taxons gestellt. Für einige potenzielle Bestäuber-Arten konnten keine Daten zur Effizienz erhoben werden, da sie während der Tests nicht an den Blüten erschienen.

2.7 Das Besucherspektrum an den Blüten von *Aechmea nudicaulis* und *Vriesea friburgensis* (Veröffentlichungen 5 und 6)

Zur Bestimmung des Besucherspektrums und des Einflusses des Habitats auf dieses Spektrum wurden Beobachtungen an Blütenständen von *Ae. nudicaulis* und *V. friburgensis* in den im

Abschnitt 2.1 beschriebenen drei Gebieten auf der Insel Santa Catarina durchgeführt. Um den Einfluss des Breitengrades zu ermitteln, wurden zusätzlich *Aechmea*-Pflanzen im Reservat Cachoeira, im Staat Paraná, untersucht. Während der Feldstudien an den Infloreszenzen wurden die Blütenbesucher so weit wie möglich bestimmt. Um eine Beeinflussung des Besucherspektrums zu verhindern, wurden die Frequenzen der einzelnen Besucherarten durch Zählen und nicht durch Abfangen der Individuen an den Blüten ermittelt.

3 Ergebnisse

3.1 Interaktionen zwischen Bromelienblüten und assoziierten Tieren

Veröffentlichung 1: *Grohme S, Steiner J & A Zillikens (2007) Destruction of Floral Buds in the bromeliad Vriesea friburgensis by the Phytophagous Larvae of the Wasp Eurytoma sp in southern Brazil (Hymenoptera: Eurytomidae). Entomologia Generalis. 30, 167-172.*

Von *Vriesea friburgensis* Mez var *paludosa* (L B Smith) L B Smith 1952 wurden die Blütenstände untersucht, da beobachtet wurde, dass sich viele Blütenknospen nicht öffneten. Im Labor fanden sich in aufgeschnittenen Knospen Larven, selten auch adulte Wespen. Die Ovarien und Antheren der Knospen waren zerstört. Die Imagines wurden als Erzwespen der Familie Eurytomidae bestimmt. Wahrscheinlich handelt es sich um eine noch unbeschriebene Art der Gattung *Eurytoma* Illiger 1807. Die Larvalentwicklung wurde im Labor verfolgt und ließ eine spezifische interorganismische Beziehung zwischen dieser pflanzen-parasitischen Erzwespen-Art und ihrem Bromelien-Wirt erkennen. Wir konnten für die hier untersuchte Wespenart den Lebenszyklus von der Eiablage bis zum Schlüpfen der adulten Wespen dokumentieren. Unsere Beobachtungen zeigen, dass die Larven entweder nach direkter Entwicklung sofort schlüpfen oder, wenn sie gegen Ende der Blühphase der Wirtspflanze heranwachsen, eine Diapause von wenigstens sechs Monaten durchlaufen. Der Lebenszyklus von *Eurytoma* sp. ist eng an den Lebenszyklus von *V. friburgensis* angepasst; ein Wirtswechsel wurde nicht beobachtet.

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Veröffentlichung 1: Destruction of Floral Buds in the bromeliad *Vriesea friburgensis* by the Phytophagous Larvae of the Wasp *Eurytoma* sp in southern Brazil (Hymenoptera: Eurytomidae)

SIMONE GROHME, JOSEFINA STEINER & ANNE ZILLIKENS

Received: 2007-05-07/2007-07-03 Accepted: 2007-07-04

Grohme S, Zillikens A [Univ Tübingen, Germany] & Steiner J [Fed Univ, Florianópolis, Brasil]: **Destruction of Floral Buds in the bromeliad *Vriesea friburgensis* by the Phytophagous Larvae of the Wasp *Eurytoma* sp in southern Brazil (Hymenoptera: Eurytomidae).** – Entomol Gener 30(2): 167–172; Stuttgart 2007-09.

In the terrestrial bromeliad *Vriesea friburgensis* Mez var *paludosa* (L B Smith) L B Smith 1952 of Southern Brazil it was observed that several flower buds did not open. The reproductive tissue at the base of the buds was destroyed by a phytophagous insect larva. The emerging adults were determined as eurytomid wasps, probably of a yet undescribed species of the genus *Eurytoma* Illiger 1807. In the laboratory, the course of its preadult development was studied, revealing a specific interorganismic relation between this parasitic hymenopteran and the bromeliad host.

Keywords: *Eurytoma* Illiger 1807 – *Vriesea friburgensis* – Bromeliaceae – flower parasitism – bud sterilisation – phytophagous wasp larvae – Southern Brazil

GROHME S, ZILLIKENS A [Univ Tübingen, Deutschland] & Steiner J [Fed Univ, Florianópolis, Brasilien]: **Zerstörung der Blütenknospen der Bromelien-Art *Vriesea friburgensis* in Südbrasilien durch die phytophagen Larven der Erzwespen-Art *Eurytoma* sp (Hymenoptera: Eurytomidae).** – Entomol Gener 30(2): 167–172; Stuttgart 2007-09.

In Südbrasilien ist häufig zu beobachten, dass sich Blütenknospen der terrestrischen Bromelie *Vriesea friburgensis* Mez var *paludosa* (L B Smith) L B Smith 1952 nicht öffnen. Am Grunde aufgeschnittener Knospen fanden sich phytophage Insektenlarven; Ovarien und Antheren waren zerstört. Die Imagines wurden als Erzwespen der Familie Eurytomidae bestimmt. Wahrscheinlich handelt es sich um eine noch unbeschriebene Art der Gattung *Eurytoma* Illiger 1807. Der Verlauf der Larvalentwicklung wurde im Labor verfolgt und ließ eine spezifische interorganismische Beziehung zwischen dieser pflanzen-parasitischen Erzwespen-Art und ihrem Bromelien-Wirt erkennen.

Schlüsselbegriffe: *Eurytoma* Illiger 1807 – *Vriesea friburgensis* Mez var *paludosa* (L B Smith) – Bromelien – Blütenparasitismus – Knospensterilisation – phytophage Wespenlarven – Südbrasilien

1 Introduction

Vriesea friburgensis Mez var *paludosa* (L B Smith) L B Smith 1952 is a common bromeliad species in secondary forest and restinga habitats in the State of Santa Catarina [REITZ 1983, DA SILVA SOUZA 2004]. Although the huge inflorescences present an ornithophilous floral syndrome [DAFNI 1992], they attract a wide range of insect flower visitors [DA SILVA SOUZA 2004]. Observations of *V friburgensis* inflorescences revealed that some flower buds remained closed and did not produce seeds. Dissection of these buds resulted in the detection of larval and adult eurytomid wasps [DA SILVA SOUZA 2004].

The family Eurytomidae comprises 88 genera and 1424 species [NOYES 2003]. The majority of the eurytomid wasps are endophytic, living as phytophagous plant parasites or as parasitoids of insects feeding on plants [NOYES 2003]. So far, two *Eurytoma* species are known to be associated with bromeliads. *Eurytoma aerflora* Bugbee 1975 is a parasitoid of *Epimorius testaceellus* Ragonot 1887 (Lepidoptera: Pyralidae), the caterpillar of which feeds on flowers of *Tillandsia fasciculata* Sw 1788 [BUGBEE 1975], whereas *Eurytoma werauhia* Gates 2004 has phytophagous larvae that feed in flower buds of *Werauhia gladioliflora* (H Wendl) J R Grant 1995 [GATES 2004]. There is, thus, a fundamental difference in the host parasitized between these two examples.

Since it remained unclear whether the eurytomid in *V friburgensis* flowers has an insectivorous or a herbivorous mode of nutrition, bud infestation, the life cycle of the wasp, and the damage caused by it in the *Vriesea* flowers was studied here as well.

2 Material and methods

2.1 Study area

Bromeliads were studied on Santa Catarina Island in Southern Brazil. At Santo Antônio de Lisboa, a secondary-forest area, *V friburgensis* occurred in miconietum-vegetation, a successional stage with low trees and bushes [QUEIROZ 1994, ZILLIKENS & STEINER 2004], whereas Joaquina beach and Campeche beach, two restinga sites, are characterized by typical vegetation on sand dunes [SIMINSKI et al 2004].

2.2 Plant biology

The inflorescences start to develop around mid October and flowering extends from mid December to end of March, the austral summer. The inflorescence consists of an up to 1.6 m high stalk carrying short branches with 10-15 flowers each (Fig 1).

COLOUR PLATE

Fig 1: Fresh inflorescence of flowering *Vriesea friburgensis* Mez var paludosa (L B Smith) L B Smith 1952 (Bromeliaceae). Pb = parasitized bud; fr = fruit.

Fig 2: *Vriesea friburgensis* Mez var paludosa (L B Smith) L B Smith 1952 (Bromeliaceae) plant with dry inflorescences after seed dispersal.

Fig 3: Dry branch of an inflorescence with undeveloped buds (pb) in contrast to two open fruit capsules (of).

Fig 4: Floral bud with larva of *Eurytoma* Illiger (Hymenoptera: Eurytomidae).

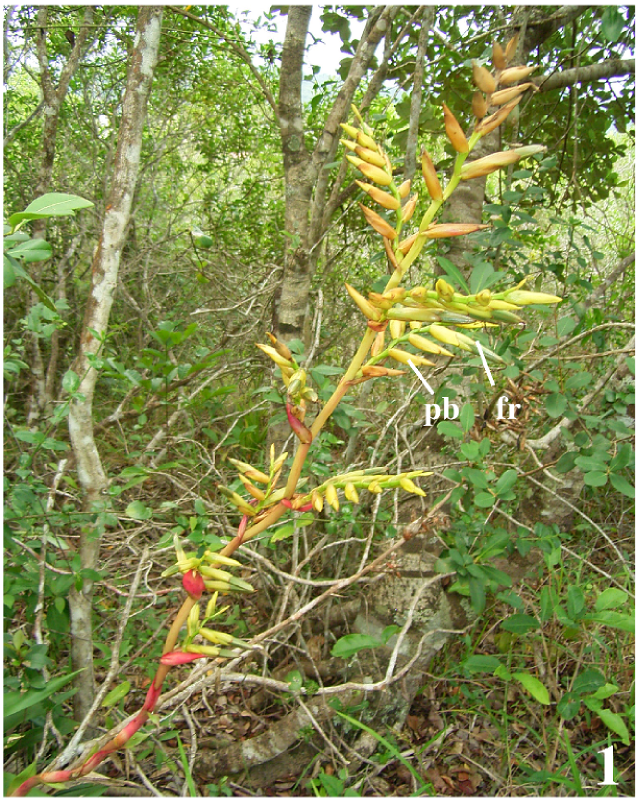
Fig 5: Aspect of *Vriesea friburgensis* Mez var paludosa (L B Smith) L B Smith 1952 (Bromeliaceae) flowers: left: intact fruit capsule (fr), right: parasitized bud (pb) with emergence hole (eh).

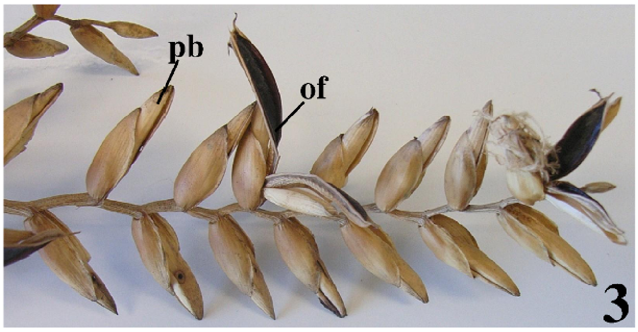
Fig 6: Female of *Eurytoma* sp (Hymenoptera: Eurytomidae) ovipositing into the flower bud of *Vriesea friburgensis* Mez var *paludosa* (L B Smith) L B Smith 1952 (Bromeliaceae).

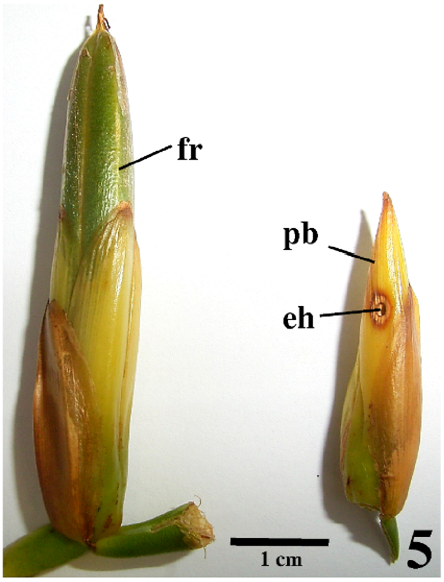
Fig 7: Inset of Fig 6.

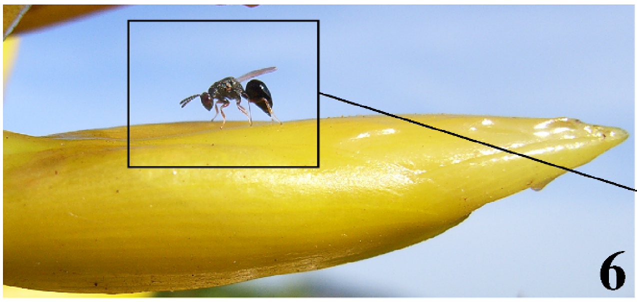
Destruction of Floral Buds in the Bromeliad *Vriesea friburgensis*

- Entmologia Generalis 30:167-172. 2007.









Mature flower buds measure 2.5–3 cm, the fruit capsule is up to 5 cm long (Fig 5). On each branch flowers open from base to top. After ripening of fruits, the dying flower stalk slowly dries up and remains standing more or less upright in the leaf rosette until the next flowering period or even longer (Fig 2).

2.3 Collection of flowers and rearing of the wasps

Branches with buds, withered flowers and developing fruits were collected on 17 March 2005. In the laboratory, one of the three sepals of buds that had not opened was cut off with a razor blade to collect the wasps inside (Fig 4). Imagines were preserved in 70% ethanol; mature larvae were placed individually in Eppendorf cups, stoppered with cotton wool, and stored at room temperature in a dark box until emergence. Developmental progress was controlled every second day between 18 March and 20 April, and every 5 days from 17 August to 20 December 2005. In addition, dry branches from the previous flowering season with buds that had not produced fruits, were collected between mid September and mid October 2006 (Fig 3). The buds were separately stored in vials and inspected daily until emergence of the wasps.

3 Results

In 2005, a few imagines and many mature larvae were obtained in March, always only one per flower. On many parasitized buds, a black spot marked the site where the female wasp had pierced the sepal during oviposition. The larva was always located in the base of the bud and the reproductive organs such as ovules and anthers had been eaten. The adult wasp gnawed a small hole of about 1 mm in diameter to leave the sterilised bud.

On 20 April, all the specimens in the Eppendorf cups were still larvae. The first two imagines of *Eurytoma* sp emerged on 17 August, eight larvae had died and the rest remained as prepupae. On 19 October 24 prepupae were counted. On 25 October there were 2 imagines, 8 pupae and 14 prepupae. Between 25 October and 27 November 4 males and 10 females emerged.

In 2006, on 19 September a few dry buds contained already fully developed imagines whereas natural emergence of 33 females and 36 males from the dry buds collected earlier that spring took place between 26 October and mid December. The overall sex ratio was 0.93 (M/F). In both years, the wasps emerged between 25 October and 11 December (Fig 8).

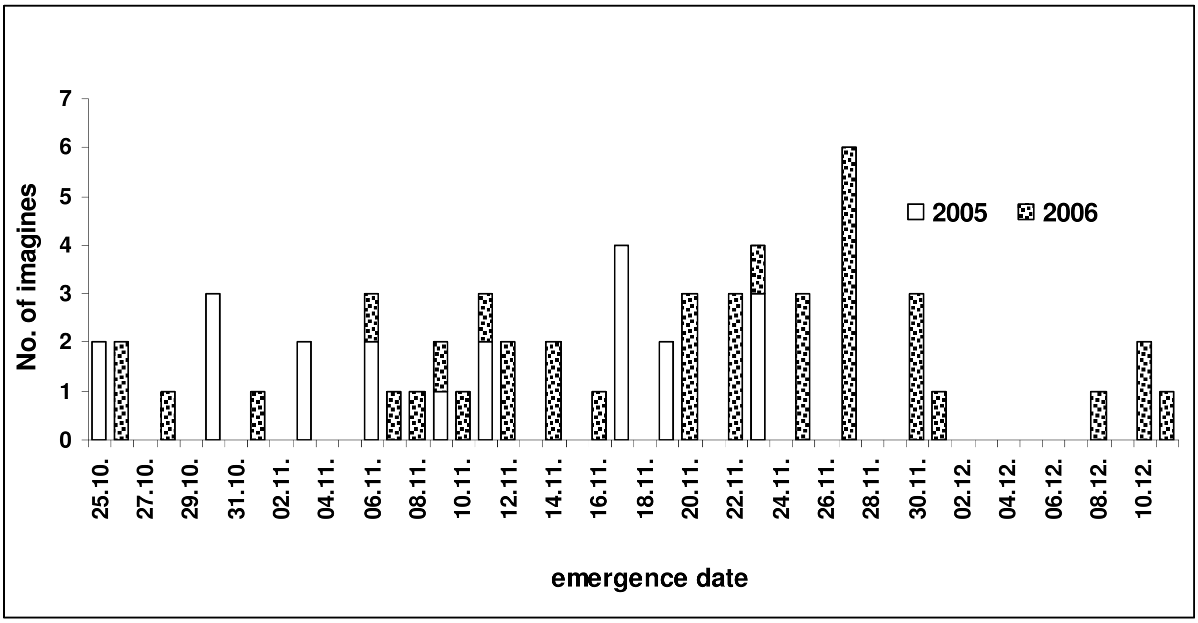


Fig 8: Emergence pattern of imagines in *Eurytoma* sp (Hymenoptera: Eurytomidae) from flower buds of *Vriesea friburgensis* Mez var paludosa (L B Smith) L B Smith 1952 (Bromeliaceae) on Santa Catarina Island, southern Brazil.

4 Discussion

This is the first record of an *Eurytoma* species with larvae parasitizing the bromeliad *Vriesea friburgensis* by feeding on the reproductive organs of the flowers. Thereby the flowers are sterilised with the consequence that the buds do not even open. It is clear that this *Eurytoma* species is phytophagous on the bromeliad flower tissues because never any evidence could be found of other insects being parasitized by this eurytomid. Also, since the flower buds had not opened and did not present any signs of damage by other predators, there is no way other potential hosts, for example caterpillars as in *Tillandsia fasciculata* Sw 1788 [BUGBEE 1975], could get into the flower buds.

Only two similar cases of eurytomids parasitizing on bromeliad flower tissues are known to date. DA SILVA SOUZA [2004] reported eurytomid wasps to parasitize the flower buds of *V friburgensis* on the mainland in southern Santa Catarina state by eating up pollen and causing the abortion of the flowers, but it remains unclear whether the ovary is also eaten, and how the larvae get into the flower buds. Furthermore, whether these wasps represent the same species of *Eurytoma* observed here, needs to be assessed by future sampling.

The larvae of *Eurytoma werauhia* also feed on the pollen – but not on the ovary – inside the flower buds of *Werauhia gladioliflora* (H Wendl.) JR Grant 1995 [GATES et al 2004] but infestation of the flowers remains obscure. While GATES et al [2004: 7] presumed that the larvae of *Eurytoma werauhia* actively search their way into the floral buds“... by an unknown manner through the calyx surrounding the corolla.”, CASCANTE-MARÍN et al [2005] reported that the eggs are laid on the flower buds and that the larvae develop inside the buds. In contrast to this, the here presented field observation of an ovipositing *Eurytoma* female (Fig 6-7) together with the punctures on the sepals clearly shows that the egg is directly placed into the flower bud.

The new results indicate that the larvae of the last generation undergo a prepupal diapause of at least 6 months, and that imagines remain inside the dry buds until the onset of the next flowering period of *V friburgensis*. This is possible due to the fact that the sterilized buds remain intact on the inflorescence stalk which does not rot but stays in place for many months or even years. Some emergence holes observed in fresh buds suggest that there may be several short cycles without diapause during the extended flowering period. Such an adaptation to the reproductive cycle of the plant can be regarded as part of an established host-parasite system and a new case of species-specific bromeliad-insect interactions.

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Strymon ziba und *S. oreala* sind bekannte Fruchtschädlinge in Ananasplantagen. Die Beziehung zwischen Bläulingen der Gattung *Strymon* und ihren Wirtspflanzen, vier Bromelien im Atlantischen Regenwald Südbrasiliens, wurde dokumentiert. Die Beobachtungen liefern Daten zum Verhalten und zum Lebenszyklus der Raupen sowie zu deren Wirtsspezifität. Feldbeobachtungen zeigten, dass Raupen der Art *Strymon ziba* sich von Früchten der Bromelie *Ae. nudicaulis* ernährten, Raupen von *S. oreala* von Früchten von *Ae. lindenii* und *Ae. caudata*, und Raupen der Bläulingsart *S. serapio* wurden an den Fruchtkapseln von *Vriesea friburgensis* beobachtet. *Strymon ziba* und *S. oreala* waren fakultativ mit mehreren Arten von Ameisen assoziiert. Zudem wurden zwei Parasiten entdeckt: aus jeweils einer Puppe von *S. ziba* und *S. oreala* schlüpften parasitische Wespen.

Veröffentlichung 2: Association of three species of *Strymon* Hübner (Lycaenidae: Theclinae: Eumaeini) with bromeliads in southern Brazil

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Abstract. As part of a project studying the species richness of bromeliad flower visitors and the diversity and nature of their animal-plant interactions, three species of the lycaenid butterfly genus *Strymon* were recorded as pests of bromeliad inflorescences. *Strymon ziba* fed on the fruits of *Aechmea nudicaulis*, *S. oreala* on those of *Ae. lindenii* and *Ae. caudata* and *S. serapio* on the dry capsules of *Vriesea friburgensis*. The caterpillars of *S. ziba* and *S. oreala*, pests of cultivated pineapple, were facultatively associated with ants. One *S. ziba* pupa was parasitized by a chalcidid wasp. One *S. oreala* pupa was parasitized by an ichneumonid wasp. Behaviour and life history data of the caterpillars are described and aspects of the host specificity of the lycaenids and potential pest control by parasitoid wasps are discussed.

Key words: *Aechmea*, animal-plant interactions, Atlantic rain forest, Bromeliaceae, Chalcididae, herbivory, Ichneumonidae, parasitism, restinga, *Vriesea*.

INTRODUCTION

Bromeliaceae, a neotropical plant family, can be considered keystone species by providing microcosms for the richness of tropical rain forests due to the high diversity of animal taxa, especially arthropods, associated with them (Frank & Lounibos, 2008). Among the latter, Lepidoptera are major herbivores with many caterpillars feeding upon bromeliad foliage: *Napaea eucharilla* Bates (Riodinidae) on *Werauhia sanguinolenta* (Cogniaux & Marchal) J. R. Grant (syn. *Vriesea sanguinolenta*, Schmidt & Zotz 2000), *Aechmea bracteata* Grisebach, *Ae. nudicaulis* (L.) Grisebach (Beutelspacher, 1972) and *Ananas comosus* (L.) Merrill (Schmidt & Zotz, 2000), *Caria domitianus ino* Fabricius (1793) (Riodinidae) on *Tillandsia caput-medusae* E. Morren (Beutelspacher, 1972; Frank & Lounibos, 2008), *Dynastor darius darius* Stichel and *D. macrosiris* Westwood (Nymphalidae, Urich & Emmel, 1991a, b) on *Ae. nudicaulis* and *Castnia boisduvalii* Walker (Castniidae, Biezanko, 1961; Frank & Lounibos, 2008) on *T. aeranthos* (Loiseleur) L. B. Smith. Beutelspacher (1972) also mentioned *Thecla hesperitis* Butler & Druce 1872 (Lycaenidae), which is a mistaken record of *Ziegleria hesperitis*, feeding on *Tillandsia caput-medusae*, but voucher specimens of "*Thecla hesperitis*" in UNAM (Universidad Nacional Autónoma de Mexico) examined by Robert K. Robbins are in fact *S. serapio* Godman & Salvin (1887) (R. K. Robbins, pers. comm.). Not only the plants' vegetative parts, but also their inflorescences contribute significantly to the local faunal biodiversity by providing resources for a great variety of flower visitors that act as pollinators or pollen and nectar robbers (Sazima & Sazima, 1999; Machado & Semir, 2006; Canela & Sazima, 2003; Schmid et al., b, submitted). In addition to causing leaf damage, some herbivorous arthropods associated with bromeliad inflorescences directly interfere with plant reproduction by feeding on reproductive tissues of flowers and fruits, like beetles, butterflies and moths, grasshoppers and even crabs (Fischer et al., 1997; Canela & Sazima, 2003; Frank & Lounibos, 2008). An example of inconspicuous herbivory affecting plant reproductive success was observed in the bromeliads *Vriesea friburgensis* Mez and *Werauhia gladioliflora* (H. Wendland) J. R. Grant whose buds are parasitized by *Eurytoma* wasps (Hymenoptera, Eurytomidae) so no fruits are formed (Gates & Cascante-Marín, 2004; Grohme et al., 2007). Beyond that, cases of seed predation by *Cholus* and *Metamasius* weevils (Coleoptera, Curculionidae) (Frank 1999) and *Epimorius testaceellus* Ragonot 1887 (Pyralidae) larvae that develop in flower pods of *Tillandsia fasciculata* Swartz (1788) (Bugbee, 1975; Heppner, 1992) have been reported. New World hairstreaks (genus *Strymon*, Lycaenidae: Theclinae: Eumaeini) use ornamental bromeliads (genera *Aechmea*, *Tillandsia*) and the commercial pineapple (*Ananas comosus*) as host plants (Robbins & Nicolay, 2002). *Strymon megarus* Godart 1824 (syn. *Thecla basilides*, also misspelled as *T. basalides*) larvae feed on *Ananas* and other bromeliads like *Ae. bracteata* (Beutelspacher, 1972; Frank & Lounibos, 2008) and can be considered pest species. *Strymon ziba, S. serapio* (Robbins & Nicolay, 2002) and *S. oreala* (Zikán, 1956), the species examined in our study, were reported to eat bromeliads, *S. ziba* Hewitson 1868 and *S. oreala* Hewitson 1868 are known pests of *A. comosus* (Harris, 1927; Zikán, 1956). Since caterpillars and other immature stages of the Eumaeini are small and cryptically coloured, food plants have been recorded for only 25% of the species (Duarte et al., 2005).

Studying the species richness of bromeliad flower visitors and the diversity and nature of their animal-plant interactions in the Atlantic rain forest of southern Brazil, we found lycaenid caterpillars attacking developing fruits. In order to assess the specificity of these associations we examined inflorescences of four common sympatric bromeliad species, *Aechmea nudicaulis, Ae. lindenii* (E. Morren) Baker, *Ae. caudata* Lindman 1891 (Bromelioideae) and *Vriesea friburgensis* (Tillandsioideae), for the presence of larvae and reared them for identification. We also recorded basic data on development, behaviour and natural enemies of the caterpillars.

MATERIALS AND METHODS

Bromeliads with inflorescences were searched for eggs and larvae between November 2006 and June 2008 at four study sites (frost-free subtropical habitats): Santo Antônio de Lisboa and the Environmental Conservation Unit Desterro UCAD (both secondary forest; 27°30'26" S, 48°30'28" W; 27°31'50" S, 48°30'50" W) (Zillikens et al., 2001; Zillikens & Steiner, 2004) as well as Joaquina Beach and Campeche Beach (dune vegetation, Sampaio et al., 2002; 27°40'38" S, 48°28'48" W; 27°37'37" S, 48°26'59" W), on Santa Catarina Island, southern Brazil. All bromeliads examined were growing terrestrially although *Aechmea nudicaulis* also occurs on trees. In total, 20 infested bromeliads of four species (*Aechmea nudicaulis*, n = 11; *Ae. lindenii*, n = 2; *Ae. caudata*, n = 5; and *Vriesea friburgensis*, n = 2), growing terrestrially on rocks, in sand or shallow soil, were taken to the laboratory. Presence, size and colour of eggs and caterpillars on the inflorescences were observed regularly every 1-2 days. Ants associated with lycaenid larvae were also collected. When larvae had finished feeding and retreated for pupation, the bromeliad plants were enclosed with fine gauze to capture the emerging adult butterflies.

Voucher specimens of the recorded butterfly, ant and parasitoid species were deposited in the entomological collection of J. Steiner at the Native Bee Laboratory (LANUFSC), BEG, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil.

RESULTS

Three lycaenid species of the genus *Strymon* were reared from the caterpillars found on four bromeliad species. All constitute new records of parasite/host association. Up to four caterpillars were found simultaneously on one inflorescence.

***Strymon ziba* (Hewitson 1868)**

We found 24 larvae of *S. ziba* on 11 inflorescences of the bromeliad *Aechmea nudicaulis*, yielding an average of 2.2 caterpillars per inflorescence (range 1-4) from November 2006 to January 2007. Seventeen adults emerged in the laboratory, overall sex ratio was 0.7 (M/F). On all inflorescences we detected small white spherical bodies, the eggs from which the larvae had hatched (Fig. 1A, B).

The colour of the larvae was cryptic and changed during their growth from whitish-yellow to reddish-pink (Fig. 1C, D). The former matched well to the fruits whereas the latter matched well to the inflorescence stem. The larvae appeared shortly after the end of the flowering period and stayed close to the ripening fruits. Larval feeding behaviour consisted of gnawing a hole into the fruit base large enough for the smaller larval stages to enter the fruit completely and for the larger stages to insert the head and anterior part into the cavity. Through this hole they fed on the soft nutritive tissues of the ovary and ovules, leaving the rigid cortical outer wall of the developing fruit mostly intact (Fig. 1A, B). On an inflorescence of *Ae. nudicaulis* with two larvae feeding, 30 fruits were damaged, resulting in a mean of 15 fruits damaged per larva. Mean fruit loss per inflorescence was 84.5% (n = 2). Occasionally, the larvae drew back from the fruits to hide under the bracts for about half a day, probably for moulting.

Development in the egg took five days (n = 2). The larval phase lasted 13-15 days (n = 1). The fully grown larvae (≈12-15 mm length) moved into the bromeliad rosette where they pupated on the upper side of the leaves half way between tip and base; one male pupated on a bract of the inflorescence. Pupation took 8-11 days (n = 3). Imagines (Fig. 1E) emerged between mid November until end of January, synchronized to the flowering/fruiting period of *Ae. nudicaulis*.

In the laboratory, caterpillars were occasionally tended by ants of four species: *Crematogaster limata* Smith 1858, *Linepithema iniquum* Mayr 1870, *Monomorium floricola* Jerdon 1851 and *Paratrechina* sp. (Fig. 1F-I).

Figure 1. *Strymon* larvae on bromeliads on Santa Catarina Island, Southern Brazil.

A-P: Strymon larvae and imagines and associated ants

A-B: Infested fruits of *Aechmea nudicaulis*, Santa Catarina Island, Brazil. **A**: Fruit with feeding hole and larval faeces of *Strymon* caterpillar and a hatched egg of *Strymon ziba* at the base of the fruit. **B**: Fruit with feeding hole and a closed egg. **C-D**: Colour change in *Strymon ziba* larvae. **C**: Small, whitish-yellow. **D**: Larger larva after colour change to reddish-pink. **E**: *Strymon ziba* female, collected on *Aechmea nudicaulis*, Santa Catarina Island, Brazil. Right-hand side ventral view, left-hand side dorsal view. **F-I**: Ants associated with *Strymon ziba* caterpillars. **F**: *Crematogaster limata*. **G**: *Linepithema iniquum* . **H**: *Monomorium* sp. (floricola). **I**: *Paratrechina* sp. **J**: *Strymon oreala*, female, right-hand side ventral view, left-hand side dorsal view. **K**: *Strymon oreala* larva feeding on fruits of *Aechmea caudata* . **L**: Larva of *S. oreala* feeding on withered flower petals. **M**:

Tapinoma melanocephalum ant on the back of a *S. oreala* larva (white arrow). **N**: *Strymon serapio*, female, right-hand side ventral view, left-hand side dorsal view. **O**: Larva of *S. serapio* on dry fruit of *Vriesea friburgensis* with feeding hole. **P**: Dry fruit of *Vriesea friburgensis* with exuvia of *S. serapio* inside. Length of exuvia: ≈ 12 mm.

Q-W: *Strymon* larvae at *A. lindenii* and parasitoids.

Q-R: *Strymon* larvae. **Q**: *Strymon* larva on *A. lindenii* feeding on fruit. **R**: *Strymon* pupa on infructescence. **S-T**: *Anisobas*, a parasitoid of *Strymon* sp. **S**: Lateral view of the *Anisobas* imago that hatched from the *Strymon* exuvia. **T**: Opened *Strymon* exuvia besides hatched *Anisobas* imago. **U-W**: *Conura*, a parasitoid of *Strymon* sp. **U**: Opened pupal case of *Strymon* sp. **V**: Imago of *Conura* sp., dorsal view. **W**: Lateral view.

Tending worker ants walked over the bodies of the caterpillars and took up small droplets secreted posterodorsally (Fig. 1F).

Additionally, we observed *S. ziba* imagines sucking extrafloral and floral nectar from inflorescences of *Ae. nudicaulis* and one female laying one single egg on each of two recently withered flowers, respectively. The female flew around the inflorescence and sucked nectar of several flowers before ovipositing. The initially greenish eggs turned white after a few minutes. Thereafter, the female left the inflorescence.

Strymon oreala (Hewitson 1868)

We discovered seven larvae of *S. oreala* (Fig. 1J) on five inflorescences of the bromeliad *Ae. caudata* (April 2008) and two larvae on two inflorescences of *Ae. lindenii* (August – September 2007). As described for *S. ziba* on *Ae. nudicaulis* larvae fed on the developing fruits of *Ae. lindenii* and *Ae. caudata* (Fig. 1K-M) and retreated into the rosette for pupation. Additionally, a larva was seen feeding on withered flower leaves, probably eating old reproductive structures inside the petals (Fig. 1L). On an inflorescence of *Ae. caudata* with one larva feeding, 15 fruits were damaged. The pupal stage took 15-16 days (n = 4). The larvae were reddish-pink like the inflorescence stem (Fig. 1 K-M). Occasionally, single ants of the species *Tapinoma melanocephalum* Fabricius 1793 were observed on *S. oreala* larvae (Fig. 1M).

Strymon serapio (Godman & Salvin 1887)

We detected four brownish-yellow larvae of *S. serapio* (Fig. 1N) on two inflorescences of the bromeliad *Vriesea friburgensis* in secondary forest in December 2007. The larvae chewed a hole into the hard capsule of the developing fruit (Fig. 1O) and fed on the seeds within.

Pupation took place inside the empty fruit capsule (Fig. 1P), the imago emerged after 11 days (n = 1).

Parasitoids

On two occasions we found *Strymon* brood infested with a parasitoid. The first case (14 November 2005, Campeche Beach) was a larva on an inflorescence of *Ae. lindenii* (Fig. 1Q). Since the only identified *Strymon* infestation of this bromeliad was by *S. oreala* (see above) we assume that the parasitized larva belonged to the same species. The larva pupated on the infructescence on 18th November 2005 (Fig. 1R). This might, however, not be the usual location for pupating because Aurum® insect glue had been applied to the infructescence stem, thus preventing the caterpillar from moving down to the rosette. After 18 days an ichneumonid wasp of the genus *Anisobas* (subfamily Ichneumoninae) emerged from the pupa (Fig. 1S, T).

In the second case, discovered 6 January 2007 at Santo Antônio, a pupa (Fig. 1U) was located at the upper margin of a leaf of *Ae. nudicaulis* (plant with infructescence). So far, we found this bromeliad species only to be infested with *S. ziba* (see above); hence we assume that the pupa belonged to this species. On 23 January 2007, a chalcidid wasp of the genus *Conura* (subfamily Chalcidinae), "most probably of the *flava* group" (Gérard Delvare, pers. comm.), emerged from the pupa (Fig. 1V, W).

DISCUSSION

Larval behaviour and host plants

Our findings constitute new host records for the associated *Strymon* species. The only lycaenids so far recorded on *Ae. lindenii* are larvae of an unidentified species of *Thecla* on Santa Catarina Island (Lenzi et al. 2006) with a similar feeding behaviour and life history data as described here for *S. oreala*. It is therefore possible that they did in fact observe larvae of a species of *Strymon*. Our observations also confirm some life history traits reported by Duarte et al. (2005) such as the cryptic coloration of the larvae which is well adapted to parts of the plants on which they move or feed. Besides the evident association of *Strymon* larvae with infructescences, they were even more selective in the sense that they fed only on the internal parts of the developing fruits, i.e. the ovaries, but not on leaves, sepals or other plant tissues.

By hollowing out the fruits they create their own shelter for feeding or even pupal chambers (in case of *S. serapio*).

We further report here the first data on the life cycle of the three species, all of which develop within about one month and without diapause. It is therefore likely that the *Strymon* species studied by us are multivoltine having several generations per year. This is in agreement with the pattern reported for other *Strymon* species in the tropics (Opler et al. 2009). The choice of hosts by ovipositing females depends on seasonal availability of fruiting plants. The bromeliads studied by us all have relatively short and seasonal fruiting periods, so that only one or two generations can develop on a given plant population. Adults emerging at the end of a flowering period have to seek for alternative hosts for egg laying. In our study, *Strymon oreala* was the only species recorded on two hosts both in the genus *Aechmea*. Interestingly, the inflorescences and flowers of *Ae. lindenii* and *Ae. caudata* are very similar in floral morphology and coloration (Kamke, pers. obs.), but *Aechmea lindenii* flowers from August to November (Dorneles et al., ms) whereas *Ae. caudata* flowers from March to June without overlap, though single plants of both *Aechmea* species can be found flowering outside the main flowering period, for example *Ae. lindenii* on more open areas in restinga sites throughout the year (Lenzi et al. 2006) and *Ae. caudata* in secondary forest in September (Kamke, pers. obs.). Nevertheless, there is a gap of several months for which we do not yet know the host plants. We know, however, that *S. oreala* does not attack the infructescences of *Ae. nudicaulis* or *V. friburgensis*, which flower between. Therefore, a switch to another host, whether bromeliad or not, must occur in *S. oreala*, *S. serapio* and *S. ziba*.

As *S. ziba* and *S. oreala* are pests of cultivated pineapple it would be interesting to further identify alternative host plant species in order to better understand under which circumstances the larvae reach pest status and to assess their damage to the crop. In this context it is also worth emphasizing our record of a possible natural enemy of *S. ziba*, a parasitoid wasp of the family Chalcididae. Its potential as natural biological control agent should be assessed by elucidating its life history, abundance and host specificity.

Association with ants

As has been reported from other lycaenid caterpillars, the larvae of *S. ziba* possess a dorsal secretory organ, the Newcomer's gland (Malicky 1970), that might exude honey-like droplets to appease ants. Of the species recorded, *Monomorium floricola* and *Tapinoma melanocephalum* are invasive ants (Delabie et al. 1995; Campos-Farinha 2005) and only occurred in the laboratory. We consider the same to be true for *Paratrechina* sp. for this species was only observed in the laboratory. *Crematogaster limata* and *Linepithema iniquum*, however, were frequently found nesting in the bromeliads or visiting their inflorescences (Rosumek et al. 2008, Schmid et al. a, ms) and were thus brought to the laboratory together with the plants taken in the field. These two, at least, may be considered associated with the *Strymon* caterpillars under natural conditions, albeit only facultatively since larvae observed in the field were mostly not tended by ants.

Diversity of the *Strymon* – bromeliad association

Our finding that four species of bromeliads were parasitized in very similar ways by *Strymon* larvae is remarkable in yet another aspect. It confirms a relatively high diversity and abundance of sympatric, even syntopic, *Strymon* species in bromeliad-rich Atlantic forest and restinga habitats of southern Brazil. Thus, the fact that three co-occurring species were recorded in studies on only four bromeliad species suggests that a thorough examination of further bromeliad inflorescences might result in the finding of more *Strymon* species and underlines the importance of these plants for sustaining a high diversity of the lepidopteran fauna in the Mata Atlântica.

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An den Blütenständen der Bromelienarten *Aechmea lindenii* und *Ae. nudicaulis* wurden häufig Ameisen beobachtet. Anhand von Verhaltensstudien sollte geklärt werden, wie eng die Ameisen mit den Blütenständen assoziiert sind. Um das Artenspektrum und die Frequenz der Ameisen an Blütenständen zu erfassen, wurden in drei Habitaten Ameisen beobachtet und gesammelt. Für *Ae. lindenii* wurden 36 Ameisenarten, für *Ae. nudicaulis* sieben Arten registriert. Am häufigsten fanden sich Arten der Gattungen *Crematogaster* und/oder *Camponotus* an den Blütenständen. Mit den Verhaltensbeobachtungen sollten zwei Hypothesen für die hohe Artenzahl von Ameisen an Blütenständen getestet werden: (1) Ameisen werden von extrafloralem Nektar angezogen oder (2) die Ameisen jagen die häufig in den Blüten vorkommenden Blütenmilben, welche durch Fressen von Pollen und Nektar die Reproduktion der Pflanzen beeinflussen könnten.

In dieser Arbeit wurde zum ersten Mal die Produktion von extrafloralem Nektar an sepalen Dornen an der Außenseite der Blüten von *Ae. nudicaulis* und *Ae. lindenii* nachgewiesen. Ameisen wurden an 88% aller Blütenstände registriert (vom Knospenstadium bis zur frühen Fruchtbildung). Im Gegensatz dazu konnten Milben erst nachgewiesen werden, wenn sich schon Blüten geöffnet hatten. In quantitativen Verhaltensexperimenten wurden Ameisen an den Blütenständen beobachtet. Diese Experimente ergaben, dass die Ameisen während 42% ihrer Aufenthaltsdauer an den Blüten dort die sepalen Dornen untersuchen. Jagd auf Milben wurde zwar auch beobachtet, war aber vergleichsweise selten. Im Labor konnte ein negativer Einfluss der Milben auf die Reproduktion der Blüten nachgewiesen werden: die sich unkontrolliert vermehrenden Milben hatten den gesamten Pollen aus den Antheren gefressen. Die Beobachtungen bestätigen die Hypothese, dass das Hauptziel der Ameisen der die Aufnahme von extrafloralem Nektar ist

Veröffentlichung 3: High diversity of ants foraging on extrafloral nectar of bromeliads in the Atlantic rainforest of southern Brazil

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In southern Brazil, we frequently observed inflorescences of two bromeliad species, *Aechmea lindenii* and *A. nudicaulis*, being visited by ants. We recorded the species spectrum and frequency of ants on inflorescences by examining more than 200 plants in three habitats to assess whether they were specifically associated to the bromeliad flowers. We recorded up to 36 associated ant species at *A. lindenii* and seven at *A. nudicaulis*, with non-plateauing species accumulation curves. Three species richness estimators (ICE, Jack2, and Chao2) indicated 40–46 species for *A. lindenii* in secondary forest. Ant species of the genera *Crematogaster* and/or *Camponotus* were always among the most frequent. To explain the association of bromeliad flowers with such a high richness of ant species we tested two hypotheses: (1) ants exploit extrafloral nectar on the flowers or (2) ants hunt for abundant hummingbird-transported flower mites that likely effect plant reproduction by feeding on nectar and pollen. After excluding all flower visitors we recorded for the first time the production of extrafloral nectar at the sepal tips of the two *Aechmea* species from early buds to the early fruiting period. Ants were present at 88% of all inflorescences during that period. In contrast, flower mites could only be found as soon as the first flowers had opened, that is, when the inflorescences had already been visited by ants for several days. Quantitative behavioural analyses of worker ants on *A. lindenii* inflorescences revealed that they spent 42% of their time inspecting the sepal tips. Preying on mites was rarely observed. We conclude that the extrafloral nectar secreted at the sepal tips constitutes the main target for a high diversity of unspecialized ants. Nevertheless, the ants might ultimately serve the bromeliads by reducing the mites' impact on plant fitness.

No sul do Brazil freqüentemente observamos inflorescências de duas espécies de bromélias, *Aechmea lindenii* e A. *nudicaulis*, sendo visitadas por formigas. Registramos as espécies de formigas e as suas freqüências nas inflorescências de mais de 200 plantas, em três tipos de habitat, para testar se elas foram específicamente associadas com flores de bromelias. Registramos até 36 espécies de formigas associadas a A. *lindenii* e sete associadas a A. *nudicaulis*, com curvas de acumulação de espécies não alcançando um platô. Três estimadores de riqueza (ICE, Jack2, e Chao2) indicaram 40-46 espécies para A. *lindenii* em floresta secundária. Espécies dos gêneros *Crematogaster* e/ou *Camponotus* sempre estiveram entre as mais frequentes. Para testar a associação de flores de bromélias com a alta riqueza de espécies de formigas nós testamos duas hipóteses: (1) formigas usam néctar extrafloral das flores ou (2) formigas atacam os ácaros muito abundantes, transportados por beija-flores, e que possivelmente interferem na reprodução das plantas por consumirem néctar e pólen. Excluindo todos os visitantes florais, registramos pela primeira vez a produção de néctar extrafloral nas pontas das sépalas das duas espécies de *Aechmea* no período de botões jovens até o início da frutificação. Formigas estiveram presentes em 88% de todas as inflorescências neste período. Em contraste, ácaros em flores foram encontrados somente assim que as primeiras flores abriram, momento em que as inflorescências já tinham sido visitadas pelas formigas há vários dias. Análises quantitativas do comportamento das formigas operárias em inflorescências de A. *lindenii* revelaram que elas ficaram 42% do seu tempo inspecionando as pontas das sépalas. Predação por ácaros foi observada raramente. Concluímos que o néctar extrafloral secretado nas pontas das sépalas constitue o principal alvo para uma alta riqueza de formigas não-especializadas. Contudo, as formigas poderiam, em última análise, beneficiar as bromélias reduzindo o impacto dos ácaros sobre as plantas.

Keywords: *Aechmea*; ant–plant interactions; behavioural analysis; hummingbird flower mites; Formicidae; species richness estimation

Introduction

The high abundance and dominance of ants in nearly all terrestrial habitats (Hölldobler & Wilson 1990; Alonso & Agosti 2000) and the obvious relevance of plants as nutrient providers make their interactions an important factor in many ecosystems. Associations between ants and plants are frequent and diverse (Hölldobler & Wilson 1990; Huxley 1991). They vary from loose, facultative associations to highly developed, specific relationships. The latter include mutualisms between ants and myrmecophytes such as species of the plant genera *Acacia* (Wheeler 1912; Janzen 1966; Heil *et al.* 2005), *Cecropia* (Janzen 1969; Schupp 1986), and *Macaranga* (Fiala *et al.* 1989, 1999; Fiala & Maschwitz 1990). Examples for facultative interactions are epiphytes facultatively growing on carton ant nests (Davidson & Epstein 1989), the non-specific use of leaf rosettes as nest sites by ants (Blüthgen *et al.* 2000), and the opportunistic exploitation of floral (Koptur & Truong 1998) as well as extrafloral nectar (hereafter EFN) (Koptur 1979, 2005). Most frequently, the function of extrafloral nectaries seems to be the attraction of animals, mainly ants, which protect the plant against herbivores (Koptur 2005).

Bromeliads (Bromeliaceae) are associated with ants in various ways (Frank & Lounibos 2008). Many ant species nest in leaf rosettes of epiphytic or terrestrial bromeliads (Blüthgen *et al.* 2000; Cogni & Oliveira 2004), and in so-called ant-gardens ants actively grow bromeliads and other epiphytes in the substrates of their nests (Huxley 1980; Kleinfeldt 1986; Davidson & Epstein 1989). Bromeliad–ant interactions also involve inflorescences. In several species of the genus *Dyckia*, and in *Deuterocohnia longipetala*, Galetto & Bernardello (1992) found extrafloral nectaries attracting ants to patrol the flowers. Moreover, there seems to be a mutualistic association between *Dyckia floribunda* and the ants that collect the sugar-rich EFN and, in return, deter herbivores from the inflorescences (Vesprini *et al.* 2003).

During studies on the diversity and interactions of flower visitors of bromeliads in the Atlantic rainforest of southern Brazil, we very frequently observed numerous ants at inflorescences of the species *Aechmea lindenii* and *A. nudicaulis*. There the ants appeared to be licking up something on the outsides of the corollas. These findings were unexpected because ants are not typical flower visitors and thus raised the question what attracted them to the inflorescences. It appeared obvious to suspect EFN as a reason for the ants' presence. However, in the field, no secretions or any morphological structures that could be taken for nectar glands were evident on the inflorescences. Therefore, another possible reason would be that ants were hunting for prey on the inflorescences. Both bromeliad species have mostly ornithophilous floral syndromes, and their flowers are visited by hummingbirds (Lenzi *et al.* 2006; Buzato *et al.* 2000). The birds are the vectors of phoretic mites associated to hummingbird flowers (Colwell *et al.* 1974; Lara & Ornelas 2002), and they actually transported mites to and from the flowers of our target plants (see Video S6 of the online supplementary material) which contained up to 400 mites/flower (median 30; VS Schmid, unpublished data). Flower mites feed on floral nectar (Colwell 1973, 1995; Colwell & Naeem 1994; da Cruz *et al.* 2007) and pollen (Colwell & Naeem 1994; Paciorek *et al.* 1995; own observations). Thereby they compete with the legitimate pollinators for the floral rewards and, by reducing the attractiveness of the flowers, could eventually reduce the pollination success.

In order to assess whether the associations of ants with the two *Aechmea* species are restricted to a few ant species or whether each bromeliad has a distinct set of associated species we performed an inventory of the ants occurring on the inflorescences. In addition, we assessed the influence of different habitat types on species spectrum and richness. To explain the occurrence of ants at the inflorescences we developed two hypotheses. The first one is that EFN produced on the inflorescences attracts the ants (EFN hypothesis). If valid we predict that the presence of ants is closely linked to the sites and period of EFN secretion. Also, the ants should spend more of their time sucking EFN than with other activities such as chasing mites. The second hypothesis is that ants are principally on the inflorescences to hunt the abundant mites inside the flowers (mite hypothesis). In this case, ant activity should coincide with the presence of mites in the flowers and consist mainly of hunting behaviour. We tested these hypotheses using several approaches: (1) by searching for EFN and its sources on *Aechmea* inflorescences; (2) by quantitative behavioural observations of ants and (3) by recording the frequency of ant and mite occurrences on inflorescences. Furthermore, we present observations on mite behaviour suggesting that mites have a considerable impact on plant reproduction.

Materials and methods

Study sites and organisms

The study was conducted August 2005 through January 2006 in the municipality of Florianópolis on Santa Catarina Island, southern Brazil. Observations were performed in the three habitat types “miconietum” (a pre-forest succession stage dominated by small trees of the genus *Miconia*), secondary forest, and “restinga” (a xerophilous vegetation formation on sand dunes; Sampaio *et al.* 2002) at five sites: (1) Environmental Conservation Unit Desterro (UCAD; 27°31′50″S, 48°30′50″W), a reserve of about 500 ha of the Federal University of Santa Catarina (UFSC), consisting of secondary forest and adjacent miconietum (see Zillikens & Steiner 2004); (2) a mountainside near Santo Antônio de Lisboa, covered with miconietum and secondary forest (see Zillikens *et al.* 2001; 27°30′26″S, 48°30′28″W); (3) Ecological Reserve Morro das Aranhas (27°28′11″S, 48°23′06″W); (4) Joaquina beach (27°37′37″S, 48°26′59″W); and (5) Campeche beach (27°40′38″S, 48°28′48″W), the latter three being low-vegetation restinga areas. The bromeliad species *Aechmea lindenii* and *A. nudicaulis* grow sympatrically on trees, rocks, and in the soil. In this study only terrestrial plants were examined. Both species are similar in their habitus (Figure S1) but differ in the morphology of their inflorescences and in flowering phenology.

Sampling of ants and mites

We recorded the presence of ant species as well as mites associated with bromeliad inflorescences by examining 250 plants of *A. lindenii* and 29 of *A. nudicaulis* along trails.

Each inflorescence was assigned to one of the following flowering phases: “buds” – only buds present; “open” – open flowers present; “withered” – all flowers withered but unripe fruits. Ants were hand-collected and preserved in 70% ethanol. Voucher specimens of all collected species were deposited in the collection of the “Laboratório de Abelhas Nativas”, UFSC, Florianópolis, SC, Brazil. For genus identification we used the keys of Bolton (1994), as well as Palacio and Fernández (2003). Species were identified by comparing specimens with the reference collection mentioned above, as well as ant collections in Itabuna, Brazil (CPDC, Jacques Delabie), São Paulo, Brazil (MZUSP, Carlos Brandão), and Karlsruhe, Germany (SMNK, Manfred Verhaagh). Additionally, we used the keys of Santschi (1923), Kempf (1959), Gonçalves (1961), MacKay (1993, 2000), and Lattke (1995). Specimens that could not be identified were assigned to morphospecies (30% of all species).

We checked each inflorescence for the presence of mites on the outer face of petals, sepals and rachis as well as inside one bud, one open, and one withered flower. Since hummingbirds usually visit all open flowers of an inflorescence (L Dorneles, personal communication; S Schmid, personal observation) we assumed the presence or absence of mites in one open flower to be valid for all flowers. We did not quantify the mites because of their small size and incessant twirling movements, and because every hummingbird visit changed the number of mites per flower by delivering some and removing others (see Video S6). A sample of mites pooled from several inflorescences was sent to experts for identification.

Analysis of ant species richness

In order to assess inventory completeness and species richness species accumulation curves (generated with the binomial mixture model by Colwell *et al.* 2004), uniques and duplicates (species that were observed only once or twice, respectively), and three nonparametric, incidence-based species richness estimators were computed: incidence-based coverage estimator (ICE), second-order Jackknife (Jack2), and Chao2. These estimators were chosen because of their good performance; other estimators (Michaelis–Menten, Bootstrap) did not stabilize. As a measure of sampling effort, we plotted species occurrences on the x-axis, as suggested by Longino *et al.* (2002): when examining an inflorescence, each ant species was recorded as one new incidence, independently from the number of individuals. Thus, depending on how many ant species were observed at a given inflorescence at a time, this inflorescence might be represented in the graph by zero to five data points. Species accumulation curves and species richness estimators were all computed using “EstimateS”

(Colwell 2005). Sample order was randomised 10,000 times without replacement. The upper frequency limit for rare species was set to 10. Detection and characterization of extrafloral nectar EFN production was assessed in three flowering plants of A. *lindenii* and two of *A. nudicaulis* (eight and four flowers, respectively). Their inflorescences were enclosed with plastic bags to prevent nectar consumption by flying animals. Ants and other non-flying arthropods were excluded by applying Aurum® insect glue to the inflorescence stems. EFN volume was measured after 2 h with graded capillaries (Brand, 5–10 µl) and sugar concentration was determined with a refractometer (Optec, Japan). Previous observations strongly suggested the sepal tips as sites of EFN secretion in *A. lindenii*. Therefore, four sepals from two flowers were dissected, critical-point dried, coated with gold/palladium, and examined by SEM (Cambridge Stereoscan 250 Mk2) for structures associated with EFN secretion.

Analysis of ant and mite behaviour

To quantify the activities of ants, a total of 208 workers of 13 species (see Table 1) were observed at 43 inflorescences bearing open flowers. To avoid pseudoreplication by sampling more than one inflorescence within the reach of the same ant colony, we selected plants at least 5 m apart from each other (mostly 10–200 m). In order to test the EFN hypothesis, we assigned activity of a focal worker ant to one of three categories: (1) “sepal tip inspection” – the worker’s head staying about 1 mm around the spine emergent from every sepal tip for about 0.5 s or longer (see Figure 1A, C; and Videos S8–S11); (2) “others” – behaviours such as contact with animals other than ants (including predation, see Video S7, showing mite hunting behaviour), activities inside open flowers, and non-explainable immobility; (3) “irrelevant” – behaviours not representing any obvious function for spending time at an inflorescence, e.g. cleaning behaviour, antennal contact or trophallaxis with conspecifics, and contact with other ant species. A statistical comparison between time spent on the categories “sepal tip inspection” and “others” was performed for four species (*Brachymyrmex coactus*, *Crematogaster limata*, *Cr. nigropilosa*, and *Solenopsis* sp. 1; total 133 workers) for which we had at least six observations per species lasting at least 2 min each. Observations of less than 2 min were not used for statistical analysis. All observations, none of which exceeded 10 min per worker, were recorded acoustically with a Panasonic IC Recorder RR-US360. Total observation time was 13 h 55 min. We computed the duration of each behavioural bout by analysing the recordings in real-time with an event recorder software (custom-made, University of Tübingen). For statistical analysis all such durations were expressed as time proportions of total individual observation time.

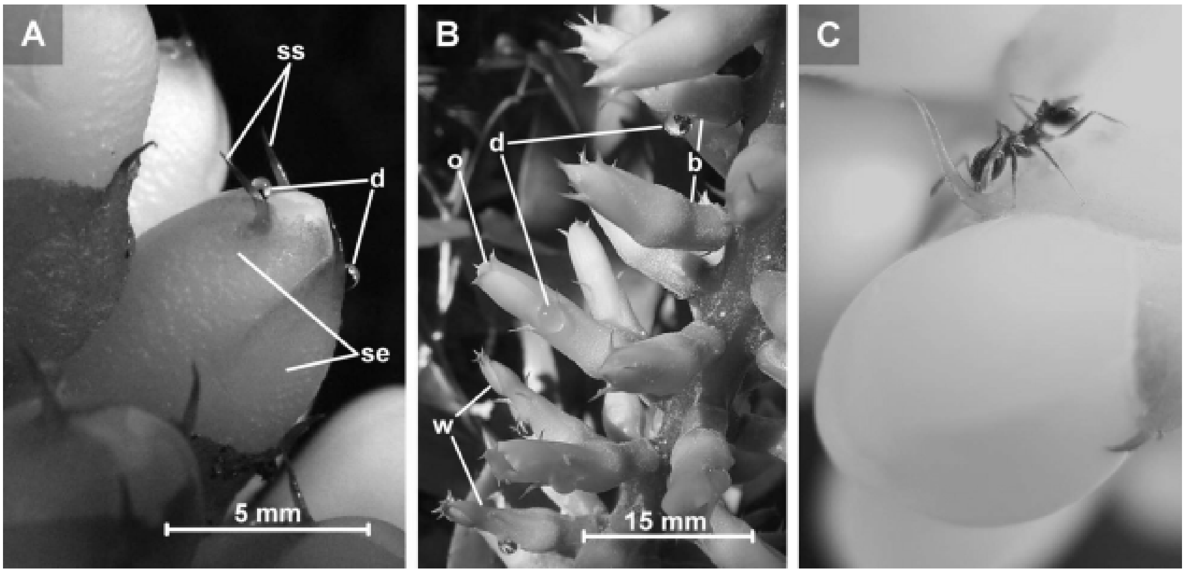


Figure 1. Extrafloral nectar at inflorescences of the two *Aechmea* species. (A) Droplets (d) secreted at the bases of sepal spines (ss) of a bud of *A. lindenii* (se, sepals); (B) droplets (d) at the outside of buds (b), open (o), and withered (w) flowers of *A. nudicaulis*; (C) worker ant (*Crematogaster limata*) inspecting a sepal spine of an *A. lindenii* bud.

To observe the behaviour and assess the influence of flower mites on pollen availability one plant of *A. lindenii* and *A. nudicaulis* with open flowers, which had been infested with flower mites, were taken into the laboratory so that hummingbirds and other flower visitors had no further access. Ants were excluded by applying Aurum® to the inflorescence stalk. After 2 d the anthers of currently open flowers infested with several hundred mites were removed at 16:00 h and checked for pollen grains at 80-fold magnification. As a control the same was done with inflorescences that had not yet had any open flowers when collected and, consequently, were free from mites.

Statistical analyses

Numbers of inflorescences with ants and mites were compared with exact contingency table analyses (Fisher's exact tests for 2 × 2 tables; Fisher–Freeman–Halton tests for larger tables) computed by the software package StatXact 8.0 (Cytel Software Corporation, Cambridge, MA, USA). Proportions of inflorescences with ants as well as with mites were compared among the flowering phases and between each pair of flowering phases, resulting in four comparisons per dataset. P values obtained from such multiple tests were adjusted by multiplication with the number of tests, conforming to the unweighted Bonferroni method (Shaffer 1995). Thus, all presented P values can be compared to the usual levels of significance. When the correction resulted in a value ≥ 1 we present $P = 0.9999$. To analyse paired behavioural data the sign test (Zar 1999) was applied. All P values were derived from two-tailed tests.

Results

Ant and mite species spectra

Altogether, we collected workers of 50 ant species from 18 genera of six subfamilies at *Aechmea* inflorescences. The species spectrum varied according to bromeliad species and habitat type (Table 1). The most frequent ant species at inflorescences of *A. lindenii* were *Brachymyrmex* sp. 3 in miconietum, *Camponotus arboreus* in restinga, and *Crematogaster limata* in secondary forest. At inflorescences of *A. nudicaulis*, *Ca. arboreus* was most frequent in restinga and *Cr. limata* together with *Linepithema iniquum* in secondary forest.

The mites belonged to at least two undescribed species of *Proctolaelaps* and *Tropicoseius* (Acari, Gamasida, Ascidae).

Table 1. Spectra and frequencies of occurrence (%, number of occurrences in parentheses) of ant species at inflorescences of the two *Aechmea* species according to habitat. obs.: number of ants used for quantitative behavioural analysis. *: subset analysed statistically.

		Aechmea lindenii			***Aechmea nudicaulis***	
ant species	**obs.**	**miconietum**	**restinga**	**secondary forest**	**restinga**	**secondary forest**
Dolichoderinae						
Dolichoderus attelaboides (Fabricius 1775)				0.8 (2)		
Linepithema gallardoi (Brèthes 1914)		2.0 (1)				
Linepithema iniquum (Mayr 1870)		2.0 (1)		5.1 (13)		25.0 (3)
Tapinoma cf. atriceps				5.5 (14)		16.7 (2)
Ectatomminae						
Gnamptogenys moelleri (Forel 1912)				0.4 (1)		
Formicinae						
Brachymyrmex admotus Mayr 1887			4.0 (1)	1.2 (3)		8.3 (1)
Brachymyrmex coactus Mayr 1887	21*	2.0 (1)		5.1 (13)		8.3 (1)
Brachymyrmex sp. 2			8.0 (2)	2.4 (6)		
Brachymyrmex sp. 3		13.7 (7)		2.0 (5)		
Camponotus arboreus (F. Smith 1858)		7.8 (4)	32.0 (8)		62.5 (10)	
Camponotus cingulatus Mayr 1862	1		4.0 (1)	1.6 (4)		
Camponotus iheringi Forel 1908				0.8 (2)		8.3 (1)
Camponotus novogranadensis Mayr 1870		2.0 (1)	8.0 (2)	2.4 (6)		
Camponotus renggeri Emery 1894			4.0 (1)			
Camponotus rufipes (Fabricius 1775)			8.0 (2)		6.3 (1)	
Camponotus trapezoideus Mayr 1870	14	3.9 (2)	4.0 (1)	7.1 (18)		

		Aechmea lindenii			*Aechmea nudicaulis*	
ant species	**obs.**	**miconietum**	**restinga**	**secondary forest**	**restinga**	**secondary forest**
Camponotus (*Myrmobrachys*) sp. 3				0.4 (1)		
Camponotus sp. 6		2.0 (1)				
Myrmelachista catharinae Mayr 1887	3			0.8 (2)		
Myrmelachista sp. 1				0.4 (1)		
Myrmelachista sp. 2			4.0 (1)			
Paratrechina guatemalensis (Forel 1885)		7.8 (4)		5.1 (13)	6.3 (1)	
Paratrechina longicornis (Latreille 1802)	4			1.6 (4)		
Myrmicinae						
Acromyrmex aspersus (F. Smith 1858)		3.9 (2)		0.8 (2)		
Cephalotes frigidus (Kempf 1960)	1			2.8 (7)		
Cephalotes pinelii (Guérin-Méneville 1844)		3.9 (2)				
Crematogaster curvispinosa Mayr 1862	5	2.0 (1)		1.2 (3)		
Crematogaster limata F. Smith 1858	117*	11.8 (6)	12.0 (3)	20.6 (52)		25.0 (3)
Crematogaster montezumia F. Smith 1858			4.0 (1)			
Crematogaster nigropilosa Mayr 1870	20*			4.0 (10)		
Crematogaster victima F. Smith 1858		5.9 (3)				
Crematogaster sp. 10				0.4 (1)		
Nesomyrmex spininodis (Mayr 1887)				0.4 (1)	6.3 (1)	
Pheidole radoszkowskii Mayr 1884		5.9 (3)				
Pheidole sp. 1				1.2 (3)		

		Aechmea lindenii			*Aechmea nudicaulis*	
ant species	**obs.**	**miconietum**	**restinga**	**secondary forest**	**restinga**	**secondary forest**
Pheidole sp. 2 group *flavens*		3.9 (2)		1.2 (3)	6.3 (1)	
Pheidole sp. 10 group *fallax*	3	5.9 (3)		1.2 (3)		
Pheidole sp. 12	3	2.0 (1)		0.4 (1)		
Procryptocerus convergens (Mayr 1887)		2.0 (1)				
Procryptocerus schmalzi Emery 1894				0.4 (1)		
Solenopsis sp. 1	13*			8.3 (21)		
Solenopsis sp. 2	3	2.0 (1)	4.0 (1)	4.0 (10)	6.3 (1)	8.3 (1)
Solenopsis sp. 3				0.8 (2)		
Solenopsis sp. 4				0.8 (2)		
Wasmannia rochai Forel 1912				6.3 (16)		
Wasmannia auropunctata (Roger 1863)				0.4 (1)	6.3 (1)	
Ponerinae						
Pachycondyla inversa (F. Smith 1858)		2.0 (1)				
Pseudomyrmecinae						
Pseudomyrmex gracilis (Fabricius 1804)		2.0 (1)				
Pseudomyrmex phyllophilus (F. Smith 1858)		3.9 (2)		2.4 (6)		
Pseudomyrmex sp. PSW05[a]			4.0 (1)			
total ant occurrences		100 (51)	100 (25)	100 (253)	100 (16)	100 (12)
number of inflorescences		24	22	198	15	17
number of ant species		23	13	36	7	7

Species richness

For *A. lindenii* and *A. nudicaulis*, we calculated ant species accumulation curves for each habitat studied (Figure 2). The highest common number of species occurrences was 12 so that a direct comparison of the five curves was only possible for the range of 1–12. Within this range the number of species increased fastest for *A. lindenii* in miconietum and slowest for *A. nudicaulis* in restinga. We plotted the development of uniques, duplicates, and the three species richness estimators together with the species accumulation curve of the ants at *A. lindenii* in secondary forest (Figure 3) because only for this sample did species richness estimators reach a plateau. Uniques peaked and clearly began to decline before the sampling ended; duplicates seemed to at least reach a plateau (Figure 3A). All three estimators stabilized at about 200 species occurrences, predicting 40–46 species. In contrast, the species accumulation curve showed an approximately logarithmic progression after about 30 species occurrences (straight line over logarithmic scale in Figure 3B) and did not deviate from it until the end of the sampling. However, the slope of the line decreased notably over a linear scale (Figure 3A).

Evidence for EFN production

On bagged inflorescences of *A. lindenii* small droplets accumulated after about 2 h, one at each sepal tip, i.e. three per flower (Figure 1A). Such droplets occurred on buds as well as on open and withered flowers (<1 µl each). Sugar concentrations ranged from 18 to 40% with a mean value of 30% (w/w). At inflorescences of *A. nudicaulis* droplets of 1–6 µl each were found hanging from the outer sepal surfaces of buds, open and freshly withered flowers (Figure 1B); sugar content ranged from 50 to 66% (mean 56%). SEM pictures of the epidermis of the sepal tips and sepal spines of *A. lindenii* did not reveal any conspicuous structures which might function as secretory organs except for stomata (see Figure S2).

Frequency of occurrence of ants and mites

Ants were present at most inflorescences (89% of *A. lindenii* and 76% of *A. nudicaulis*). Up to five species were recorded simultaneously at the same inflorescence, but one species was most frequently found (Figure 4). The distribution of ant species numbers as shown in Figure 4 did not significantly differ among the flowering phases in both bromeliad species (Fisher–Freeman–Halton tests; *A. lindenii*: P = 0.2678, n total = 247; *A. nudicaulis*: P = 0.3071, n total = 31; Table S13). The frequencies of inflorescences on which both ants and mites were present differed with the flowering phase. They rarely occurred together at inflorescences with buds only but were frequently found together at those in the "open" phase. After all flowers had withered, the co-occurrence of ants and mites decreased to around 25% (Figure 5). In both bromeliad species the proportions of inflorescences with ants did not differ significantly among flowering phases (Table 2). The same was true for mites in *A. nudicaulis*.

In contrast, mite proportions in *A. lindenii* showed significant differences among the flowering phases as well as between each possible pair of flowering phases (Table 2), which means that mites were significantly most frequent at inflorescences phase "open", followed by "withered" and "buds".

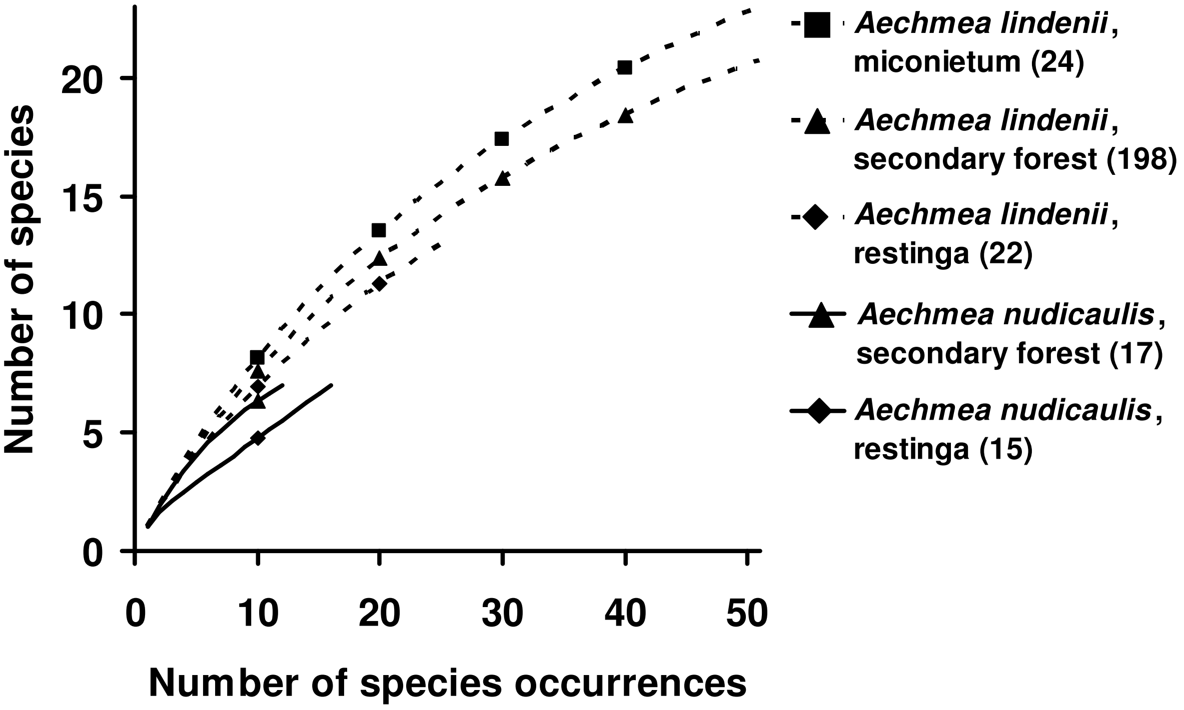


Figure 2. Species accumulation curves for visiting ants at inflorescences of *Aechmea lindenii* in three habitat types and *A. nudicaulis* in two habitat types. Numbers of sampled inflorescences are given in parentheses. The *x*-axis was restricted to 51 species occurrences for better comparability (the complete curve for *A. lindenii* in secondary forest is shown in Fig. 3).

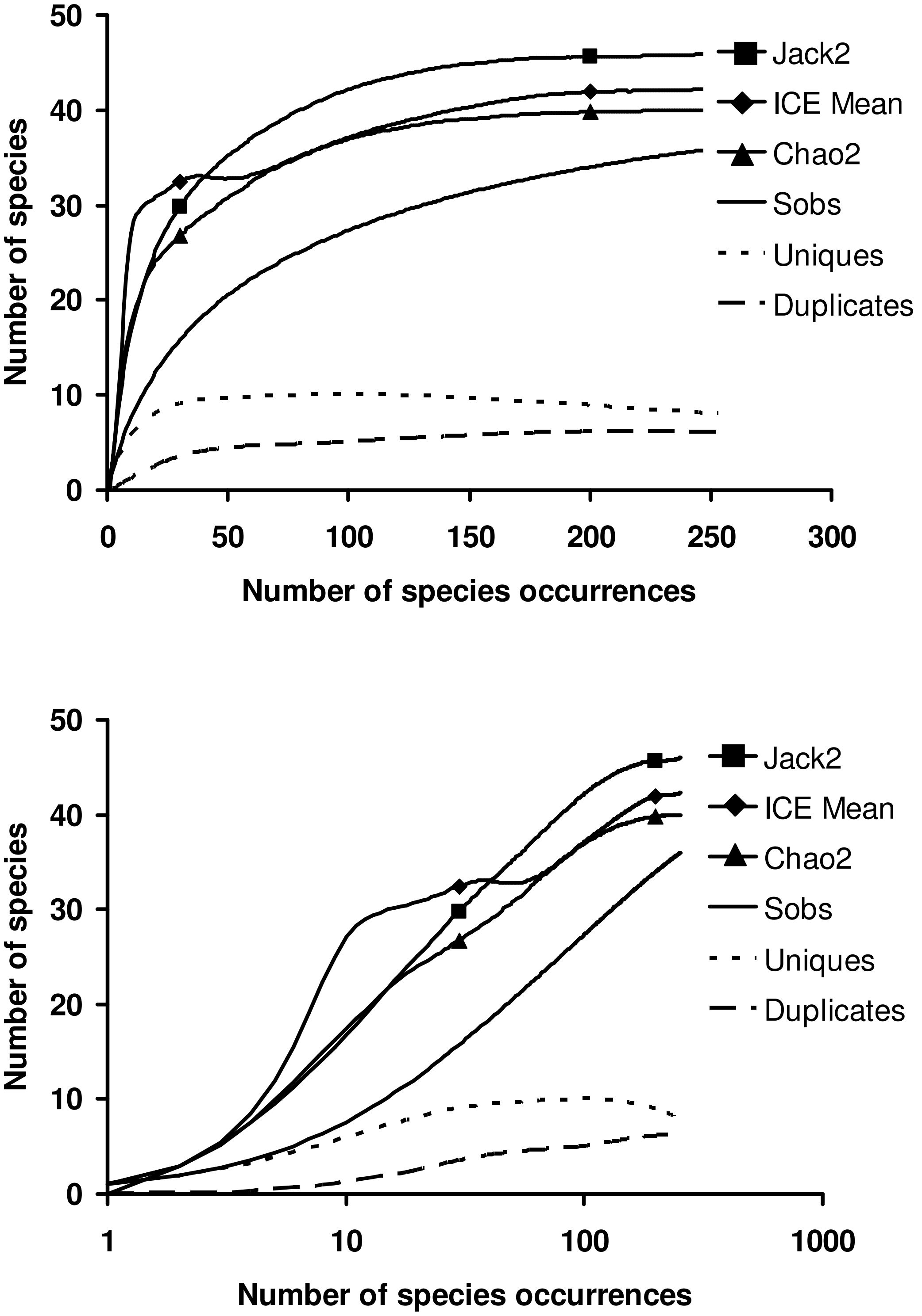


Figure 3. Species accumulation curve (Sobs = number of species observed) for *Aechmea lindenii*, secondary forest, and development of three non-parametric species richness estimators' predictions: second-order Jackknife (Jack2), ICE, and Chao2. Uniques and duplicates are shown additionally. A: x-axis is scaled arithmetically; B: x-axis is scaled logarithmically.

Behavioural analysis

The category "sepal tip inspection" made up 42% of the behaviour time of ants we observed (median for all observed workers; inter-quartile range: 27–53%). During most of the remaining time (median: 49%; inter-quartile range: 25–58%) the ants were running on the inflorescence, mainly moving between sepal tips. In four ant species, they spent significantly more time in "sepal tip inspection" behaviour than in other relevant behaviour types (Figure 6). Three workers of *Cr. limata* (out of 208 ants examined, 117 of *Cr. limata*) were observed to successfully catch and transport mites down the inflorescence, spending 12–64% of their time with those actions. Aside from our systematic observations, we once recorded a worker of *Solenopsis* sp. carrying a flower mite and a worker of *Tapinoma* sp. 1 hunting mites in the field as well as workers of *Tapinoma melanocephalum* transporting flower mites down inflorescences in the laboratory.

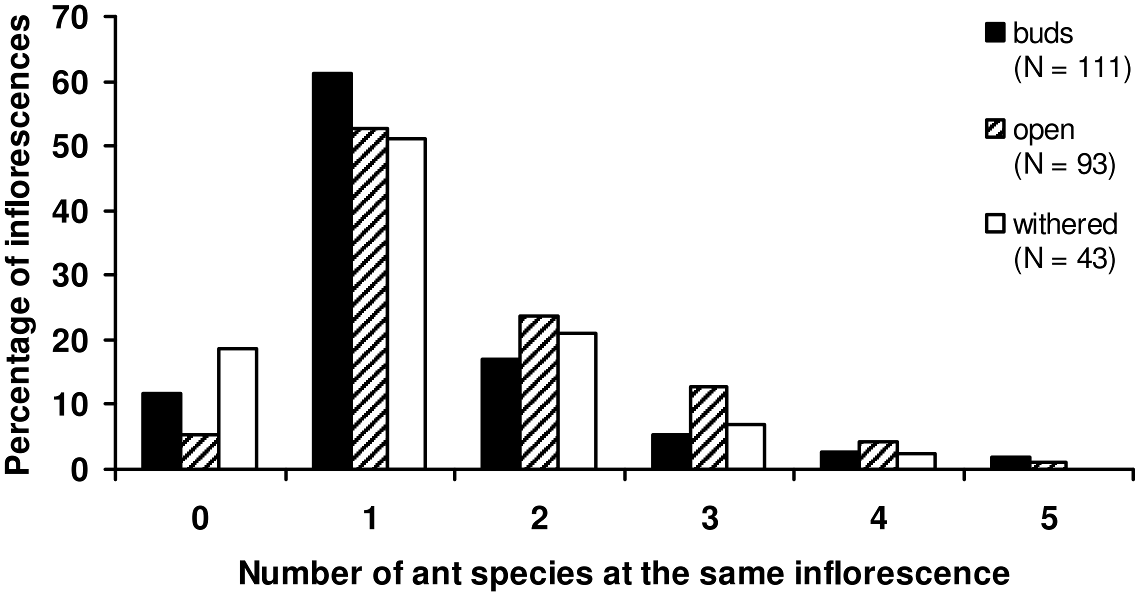


Figure 4. Percentages of *Aechmea lindenii* inflorescences with 0-5 ant species simultaneously present at a time, given for each flowering phase. N: number of examined inflorescences.

Figure 5. Frequencies (in %) of ant and mite occurrence at inflorescences of *Aechmea lindenii* and *A. nudicaulis*, according to flowering phase. N: number of examined inflorescences.

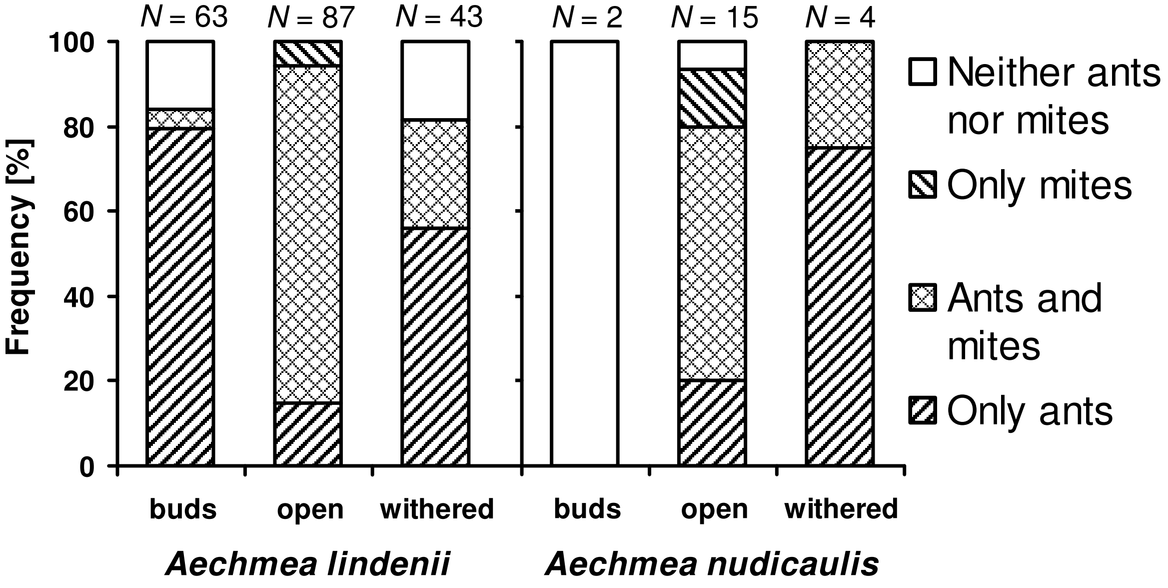


Table 2. *P* values (Bonferroni-corrected for fourfold testing) for comparisons of proportions of inflorescences with ants and of inflorescences with mites in both bromeliad species.

	***Aechmea lindenii* (193)**		***Aechmea nudicaulis* (21)**	
Compared flowering phases	**ants**	**mites**	**ants**	**mites**
buds-open-withered	0.1602	< 0.0001*	0.3000	0.2266
buds-open	0.2164	< 0.0001*	0.2941	0.4412
buds-withered	0.9999	0.0113*	0.2667	0.9999
open-withered	0.1202	< 0.0001*	0.9999	0.4696

Note: If Bonferroni correction yielded a value ≥ 1 we present $P = 0.9999$. All values were obtained from exact contingency table analyses (Fisher's exact and Fisher-Freeman-Halton tests). The numbers of inflorescences examined are given in parentheses. *Significant differences ($P < 0.05$).

Behaviour of the flower mites

The anthers from flowers with mites to which no other visitors had been admitted were almost void of pollen at 16:00 h (Figure S3). Under laboratory conditions, i.e. with plenty of food and without predators, mites proliferated rapidly within the flowers, so that extreme population densities were reached. At the end of the flowering period of the inflorescences mites were forced to occupy also the outside of the flowers sitting in several layers above each other (Figure S4). Mites readily reacted to an approaching finger by moving towards it (Video S12). During the day, mites stayed inside the flowers' corollas and were rarely seen outside. After anthesis, at around 18:00 h, the mites that remained after the last hummingbird visitor left the corolla of the withering flowers, walked to the flower buds that would open the next morning, and hid under the sepals until the onset of the next anthesis (Figure S5).

Discussion

Patterns of ant species richness on bromeliad inflorescences

The differences between the species accumulation curves indicate that both bromeliad species and habitat type influence the richness of associated ant species. Within the same habitat type, *A. lindenii* appears to be attractive to a higher number of ant species than *A. nudicaulis*, even though the latter produces larger EFN droplets with higher sugar concentrations. Characteristics of EFN, e.g. amino acid composition, might also influence ant diversity (González-Teuber & Heil 2009), so its attractiveness and chemical composition should be assessed to test this trend. Within the same bromeliad species, inflorescences in secondary forest are apparently visited by a higher number of ant species than in restinga habitats. This reflects the generally higher ant diversity of the study area's secondary forests compared to restinga: at the UCAD and Santo Antônio at least 124 species were found (Rosumek *et al.* 2008), whereas in Joaquina Beach, Bonnet & Cortês Lopes (1993) recorded only 33 ant species.

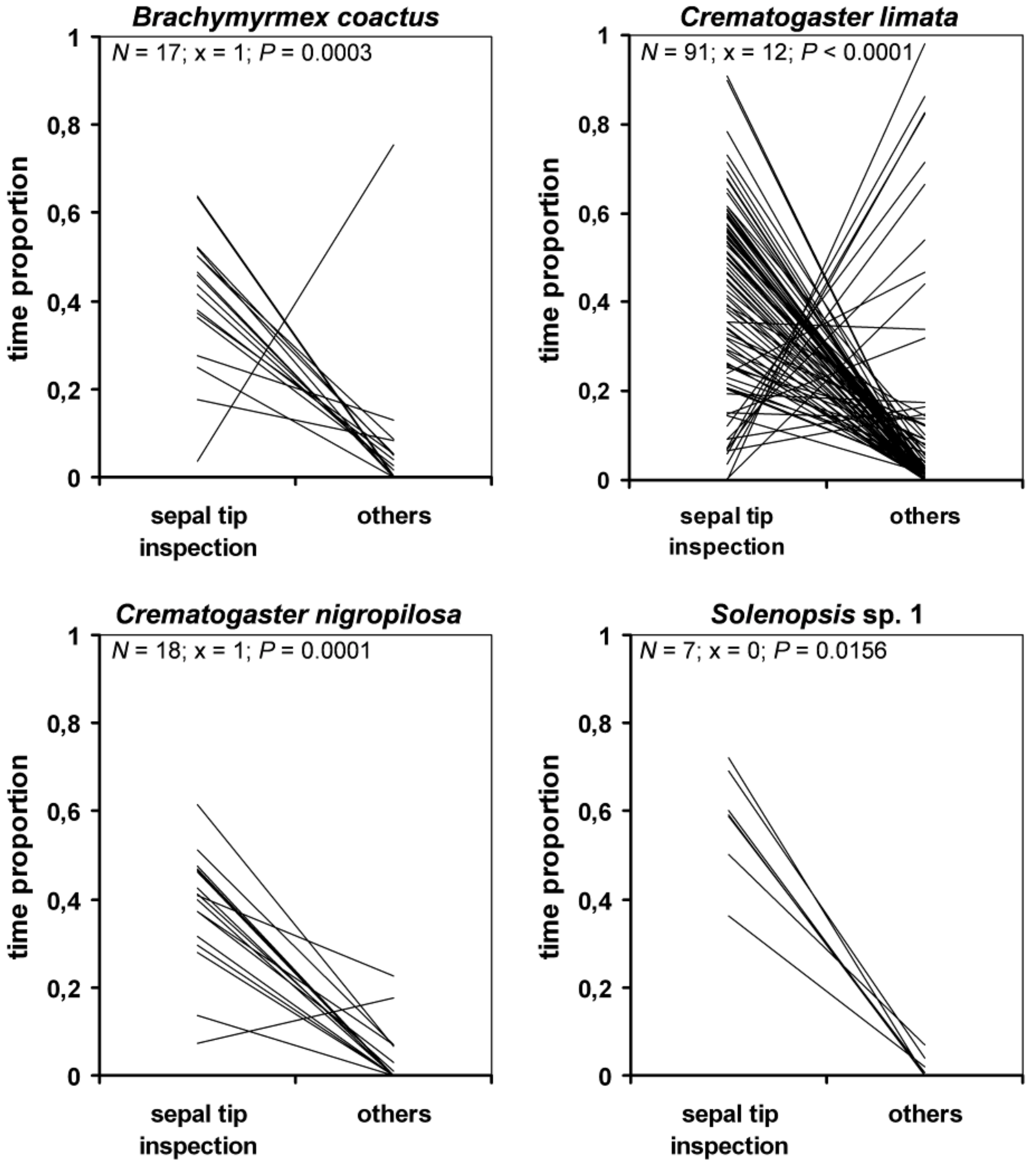
Our inventory of those associated ant species seems to be not far from complete for *A. lindenii* in secondary forest because of the beginning decline of at least the uniques, and since the observed number of species amounts to 78–90% of the species richness predicted by asymptotically performing estimators. The other inventories are far from complete, as both their species accumulation curves and the species richness estimators did not reach a plateau. The species richness values that we report, especially for *A. lindenii* in secondary forest (36 species, 40–46 estimated) and miconietum (23 species), are relatively high in comparison with other studies. Rico-Gray & Oliveira (2007: Appendix 6.1) provide an extensive bibliography on studies about EFN-associated ants. Most of the numbers of ant species presented there are far below our results. The only listed study with a higher species count per plant is that of Rickson & Rickson (1998) but they sampled over a large geographical range, and the number of ant species per country did not exceed 24. At least three studies that report a substantial diversity of EFN-associated ants provide species lists with occurrence data that we used for comparison with our inventory. Both accumulation curves and estimations based on the recomputed data of Schemske (1982), Smiley (1986), and Oliveira *et al.* (1995) lay beneath those for *A. lindenii* in secondary forest and miconietum (VS Schmid, unpublished data). Altogether, *A. lindenii* appears to attract one of the highest numbers of ant species to its extrafloral nectaries reported so far.

Spectrum of bromeliad-associated ants

The majority of ant species we observed in the various habitats occurred only rarely at the bromeliad inflorescences, and the species spectrum changed with habitat type. This indicates that most – if not all – ant species are not specialized on the studied plants but forage on them in an opportunistic manner, exploiting mainly the EFN. Because the two bromeliad species flower only from September to December (Schmid *et al.* 2010, personal observation), and thus EFN secretion is limited to only a few months, the ants cannot rely on this food source throughout the year.

Most of the ant genera in our inventory of secondary forest have been found to be associated with bromeliad rosettes in the Atlantic Forest of Santa Catarina (Rosumek *et al.* 2008), but three, namely *Paratrechina*, *Cephalotes*, and *Nesomyrmex*, are new records for secondary forest habitats in that region. Similarly, we can add at least four species (two *Camponotus* spp., one *Crematogaster* sp., and *Nesomyrmex spininodis*) to the list of ants in restinga habitats on Santa Catarina Island published by Bonnet & Cortês Lopes (1993).

Species of *Camponotus* and *Crematogaster* as well as of other genera found in this study (e.g. *Pseudomyrmex*, *Paratrechina*, *Solenopsis*) are frequently associated with extrafloral nectaries (Rico-Gray & Oliveira 2007: Appendix 6.1). In secondary forest and miconietum we often found colonies of *Cr. limata* in leaf rosettes of the two *Aechmea* species (VS Schmid, personal observation), providing an additional explanation for the high frequency of this ant species.


Brachymyrmex coactus

Crematogaster limata

Crematogaster nigropilosa

***Solenopsis* sp. 1**

Figure 6. Time proportions of behavioural categories ”sepal tip inspection“ and ”others“ for worker ants of four species at *Aechmea lindenii*. Proportions are shown as end points of lines each of which connects the corresponding values of one individual, respectively. Results of the sign test are given in each diagram. N: number of observed ants. x: test statistic.

Extrafloral nectar at bromeliad flowers

Although Lenzi *et al.* (2006) already suspected that the bromeliad *A. lindenii* produced EFN, we present here the first hard evidence for the production of EFN in *A. lindenii* and *A. nudicaulis*. We also determined that the sites of secretion were located at the sepal spines and the region around their bases. This finding is similar to the conclusions of Galetto & Bernardello (1992) who detected nectar secretion on the exterior of flowers of bromeliads in the genera *Dyckia* and *Deuterocohnia*. In *Tillandsia* extrafloral nectaries are located "on pedicels, peduncles, or stems of inflorescence" (Koptur 1992), that is, also close to flowers. Because of the absence of any specialized, gland-like structures Galetto & Bernardello (1992) concluded that the EFN is probably secreted through stomata. The same is evident for *A. lindenii* in which we found only stomata in the epidermis of the sepals. This pattern may also be valid for *A. nudicaulis* where no conspicuous structures are obvious. Therefore we categorize the extrafloral nectaries of *A. lindenii* and *A. nudicaulis* together with those of *Dyckia* and *Deuterocohnia* as formless nectaries ("gestaltlose Nektarien") without macroscopic characteristics according to Zimmermann (1932) (see also Elias 1983).

Why are ants attracted to the bromeliad inflorescences?

Our hypotheses about the reasons for the presence of ants on the *Aechmea* inflorescences are not mutually exclusive. Indeed, besides the exploitation of EFN, we observed chasing and captures of hummingbird flower mites by no less than four ant species. However, mites were almost exclusively present in open and recently withered flowers. Therefore, the existence of mites does not sufficiently explain the occurrence of high numbers of ants on inflorescences with buds only which are characterized by the absence of flower mites. Also, as predicted by the EFN hypothesis, ants spent significantly more time with sepal tip inspection than with preying on mites. Accordingly, ants were only rarely seen capturing mites, at least during daytime. Yet the occasional feeding on mites might supplement the ants' diets with nutrients lacking from nectar. At night, when the mites move on the inflorescences to new buds and hideouts (as also observed by Dobkin 1990 for mites on *Heliconia trinidatis*), they might be more exposed to ant predation, but this needs to be examined in further observations. Our results clearly favor the EFN hypothesis. The apparent preference for EFN may be explained by its good accessibility and sugar concentrations even higher than those of floral nectar (*A. lindenii*: 30% versus 27%, Lenzi *et al.* 2006; *A. nudicaulis*: 56% versus 23%, S Schmid, unpublished data), whereas capture of the very agile mites (see Video S7) and collection of floral nectar could be associated with notably more effort.

Do the ants contribute any benefits to the bromeliads?

In the absence of predators and competitors at the flowers, mites became extremely abundant, a situation rarely observed in the field, and entirely depleted the anthers of pollen. These findings suggest that flower mites are able to interfere considerably with sexual reproduction in the two *Aechmea* species. Therefore, removal of the mites by predators such as ants should be beneficial for plant reproduction. Indeed, for the Argentinean bromeliad *Dyckia floribunda* Vesprini *et al.* (2003) reported a higher number of seeds/plant when ants had access to the inflorescences than when they were excluded, but they did not mention which herbivores were attacked by the ants. The secretion of EFN attracting a wide spectrum of ant species patrolling on the inflorescences might be a means to keep populations of herbivores, especially flower mites, at a relatively low level. Thus, ants might well provide some benefits for the plants by reducing the costs that result from pollen loss caused by flower mites that are inevitably associated with hummingbird pollination. However, the herbivores' impact on the reproductive success of bromeliads has not been quantified nor is it known whether they are deterred by the ants.

Obviously, there are many open questions regarding this ant–bromeliad association system such as how mites affect the plants' reproductive fitness, and if so which other herbivores may be attacking the bromeliad inflorescences. Together with the high number of associated ant species these questions demonstrate the importance of bromeliads as hotspots of biodiversity and interactions in the Neotropics and underline the need for conservation efforts, especially accounting for this plant group.

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Online supplementary material

Additional supporting information may be found in the online version of this article:

- Figure S1. Habitus photographs of the bromeliad species studied with details of inflorescences.
- Figure S2. SEM micrographs of sepals of an *Aechmea lindenii* flower.
- Figure S3. Full and empty anthers, depending on mite presence.
- Figure S4. Flowers of *Aechmea lindenii*, occupied by mites.
- Figure S5. Flower mites on a bud of *Aechmea nudicaulis* at 23:30 h. They hide under the sepals, waiting for the flower to open.
- Video S6. Hummingbird (*Thalurania glaucopis*, female) visiting a flower of *Aechmea lindenii* and transporting flower mites on its bill.
- Video S7. *Crematogaster limata* worker hunting mites.
- Videos S8–S11. Video captures of four ant species inspecting sepal tips of *Aechmea lindenii*.
- Video S12. Reaction of flower mites on an approaching pencil and finger.
- Table S13. Numbers of inflorescences with zero to five ant species simultaneously present.

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Supplementary Material:

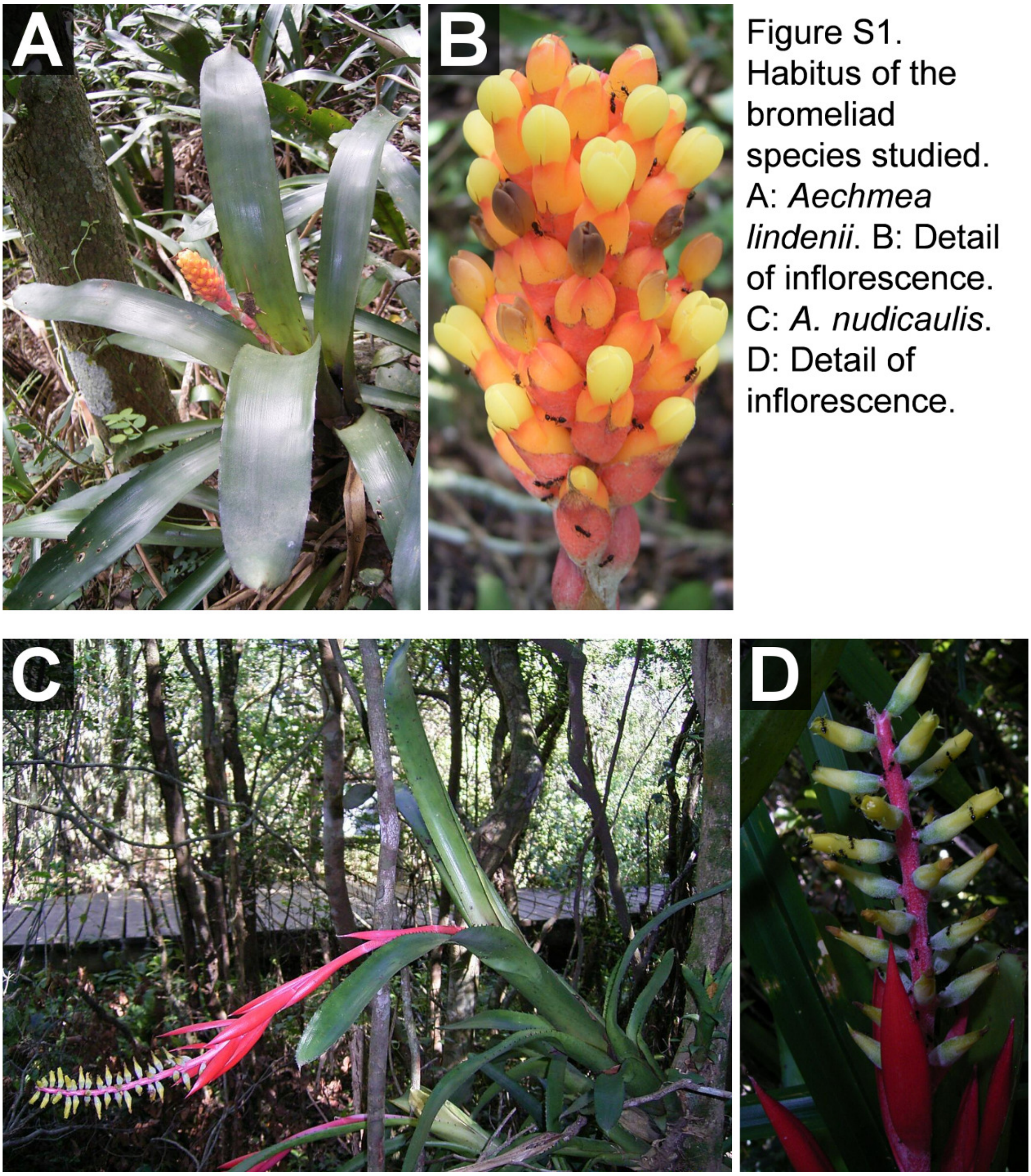

Figure S1. Habitus of the bromeliad species studied. A: *Aechmea lindenii*. B: Detail of inflorescence. C: *A. nudicaulis*. D: Detail of inflorescence.

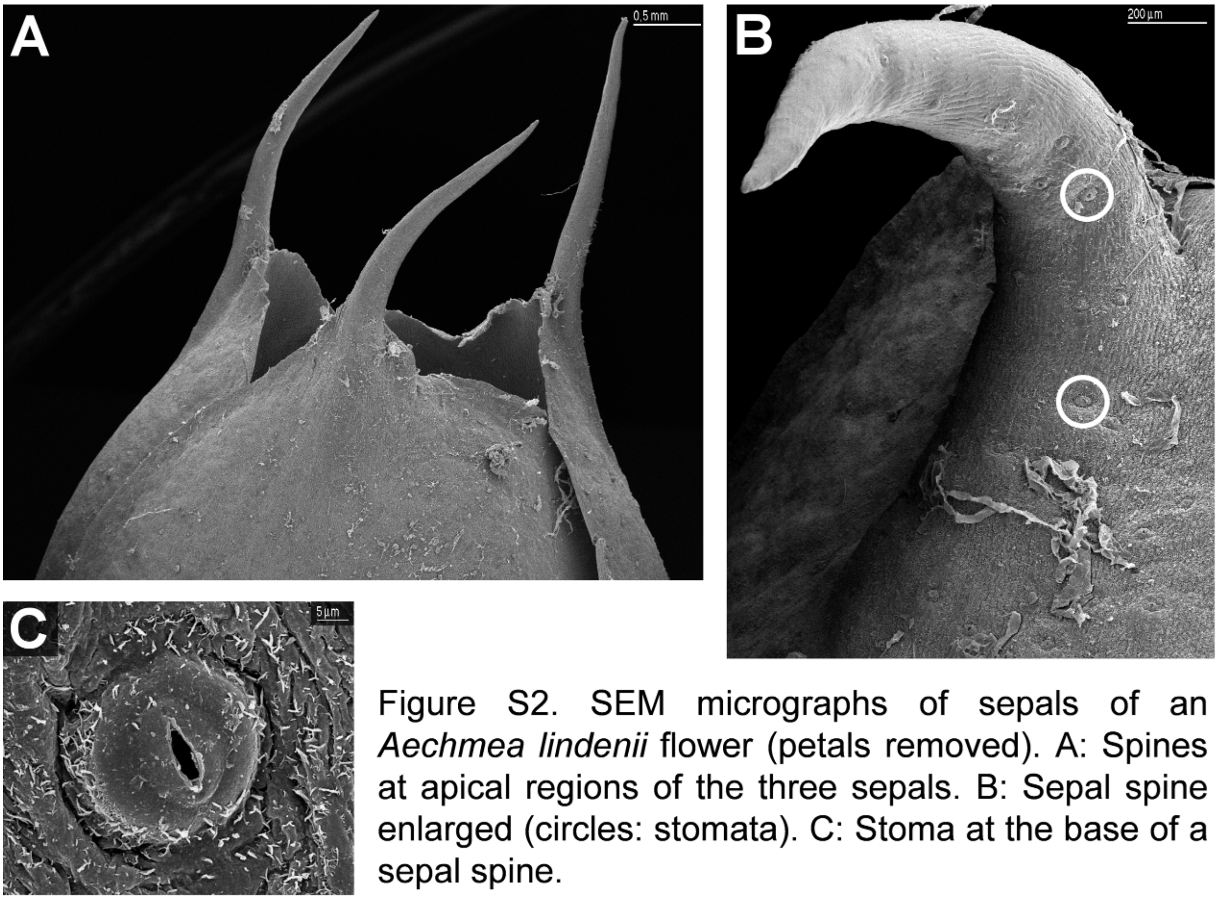

Figure S2. SEM micrographs of sepals of an *Aechmea lindenii* flower (petals removed). A: Spines at apical regions of the three sepals. B: Sepal spine enlarged (circles: stomata). C: Stoma at the base of a sepal spine.

Figure S3. Condition of open anthers of *Aechmea lindenii* (above) and *A. nudicaulis* (below) flowers at 16:00 h; on the left without mites, ants and other flower visitors; on the right with high density of mites, but without ants and other flower visitors. The number of pollen grains/flower (with six anthers) in freshly dehisced anthers is 157,000-200,000 for *A. lindenii* (Lenzi et al. 2006; Livia Dorneles & Birgit Harter-Marques pers.com,) and 82,000 for *A. nudicaulis* (Birgit Harter-Marques, pers. comm.).

Figure S4. Flowers of *A. lindenii*, occupied by mites. Towards the end of the flowering period mite populations can grow so large that many mites have to stay outside the flowers, both in the field (above) and, to a larger extent, in the laboratory in the absence of ants (below).

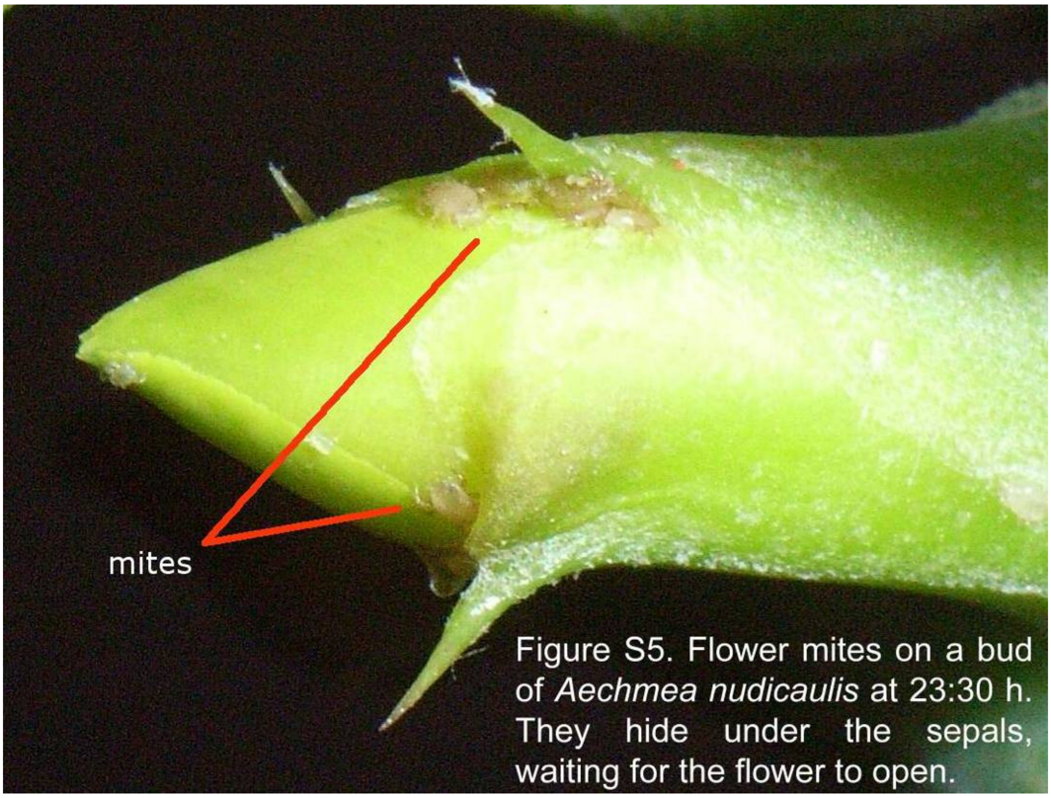

Figure S5. Flower mites on a bud of *Aechmea nudicaulis* at 23:30 h. They hide under the sepals, waiting for the flower to open.

Table S13. Numbers of inflorescences with 0-5 ant species simultaneously present. These contingency tables were analysed with Fisher-Freeman-Halton tests, yielding no significant difference among the flowering phases (*A. lindenii*: *P* = 0.2678; *A. nudicaulis*: *P* = 0.3071). The total numbers of inflorescences examined are given in parentheses.

***Aechmea lindenii* (247)**

flowering phase	**number of ant species**					
	0	1	2	3	4	5
buds	13	68	19	6	3	2
open	5	49	22	12	4	1
withered	8	22	9	3	1	0

***Aechmea nudicaulis* (31)**

flowering phase	**number of ant species**			
	0	1	2	3
buds	2	0	0	0
open	5	11	7	1
withered	0	3	2	0

3.2 Die Blütenbiologie und die effizienten Bestäuber von *Aechmea nudicaulis* und *Vriesea friburgensis* sowie die Diversität und Häufigkeit von Blütenbesuchern

Veröffentlichung 4: *Schmid S, Schmid VS, Zillikens A, Harter-Marques B & J Steiner (published online) Bimodal pollination system of the bromeliad Aechmea nudicaulis involving hummingbirds and bees. Plant Biology.* doi:10.1111/j.1438-8677.2010.00348.x

In der vorliegenden Studie wurde im Atlantischen Regenwald in Südbrasilien die Blütenbiologie von *Aechmea nudicaulis* (L.) Grisebach untersucht und die Blühperiode aufgenommen. Diese erstreckte sich von Mitte September bis Ende Dezember. Die Experimente zur Reproduktion zeigten, dass *Ae. nudicaulis* obligat xenogam und somit von Bestäubern abhängig ist. Mittels HPLC durchgeführte Nektaranalysen ergaben eine Nektarproduktion von 31,84 µl/d; die mittlere Zuckerkonzentration lag bei 23,2%. Sowohl das Nektarvolumen als auch die Konzentration waren in den ersten Stunden der Anthese am höchsten. Die Nektarproduktion und die meisten anderen Blütenmerkmale passten zu einem ornithophilen Syndrom, allerdings wurden neben den Kolibris auch eine Vielzahl von Arthropoden als Besucher registriert.

Um die Effektivität der Bestäubung durch besuchende Vögel und Insekten zu vergleichen, wurde die Samenproduktion als Folge eines einzigen Blütenbesuchs untersucht. Der Kolibri *Thalurania glaucopis* erzeugte den höchsten Samenansatz pro Einzelbesuch und war somit der effektivste Bestäuber. Diese Studie zeigte außerdem, dass auch Bienen aufgrund ihrer hohen Besuchsfrequenz funktionelle Bestäuber von *Ae. nudicaulis* sind. Das Bestäubungssystem dieser Art ist also bimodal.

Die blütenbesuchenden Bienenarten mussten differenzierter betrachtet werden, da ihre jeweilige Effizienz von der Größe und dem Verhalten der Individuen an den Blüten abhängig ist. Große Bienen haben längere Zungen als kleine und schmale Bienen. Diese können wiederum aufgrund ihrer Größe in die Blüten hineinkriechen. Arbeiterinnen sozialer Bienen kommen meistens in größeren Mengen an den Blüten vor als solitäre. Bienen, die zu groß sind, um in die Blüten zu kriechen, deren Zungen jedoch nicht lang genug sind, um den Nektar zu erreichen, werden manchmal zu Pollenräubern. Dies ist der Fall bei *Trigona spinipes*. Bienen der Gattung *Plebeia* sind zu klein, um beim Sammeln Pollen zu übertragen. Die Prachtbiene *Euglossa annectans* hat eine sehr lange Zunge, so dass sie damit den Nektar am Blütengrund erreichen kann, ohne dass ihre behaarten Körperpartien (Kopf, Thorax) die reproduktiven Blütenorgane berühren. Ähnliches ist auch bei den Schmetterlingen der Fall, die ihren Rüssel nach dem Blütenbesuch reinigen. Kurzlebige Schmetterlinge trinken nur Nektar, langlebige Schmetterlinge aus der Gruppe der Heliconiiden sammeln außer Nektar auch Pollen.

Veröffentlichung 4: Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees

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Keywords Bromeliaceae; energy supply; floral syndrome; nectar composition; pollinator effectiveness; self-incompatibility; visitation frequency.

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ABSTRACT

In order to compare the effectiveness of birds and insects as pollinators, we studied the floral biology of the bromeliad *Aechmea nudicaulis* (L.) Grisebach in the biome of the Atlantic rain forest, southern Brazil. On Santa Catarina Island, flowering extends from mid-September to the end of December, with diurnal anthesis. The reproductive system is obligatory xenogamy, thus pollinator-dependent. Flowers secrete 31.84 µl of nectar per day, with a mean sugar concentration of 23.2%. Highest nectar volume and sugar concentration occur at the beginning of anthesis. Most floral traits are characteristic for ornithophily, and nectar production appears to be adapted to the energy demand of hummingbirds. Continued secretion of the sucrose-dominated nectar attracts and binds visitors to inflorescences, strengthening trapline foraging behaviour. Experiments assessing seed set after single flower visits were performed with the most frequent visitors, revealing the hummingbird *Thalurania glaucopis* as the most effective pollen vector. In addition, bees are also functional pollinators, as substantiated by their high visitation frequency. We conclude that this pollination system is bimodal. Thus, there is redundancy in the pollination service provided by birds and bees, granting a high probability of successful reproduction in *Ae. nudicaulis*.

INTRODUCTION

Pollination syndromes are sets of floral traits that include morphological features (flower size, shape, colour), rewards (nectar, pollen) and flowering phenology (e.g. seasonal, diurnal) displayed by flowers. These sets are regarded as being adapted to attract and utilise flower visitors as pollinators (Fenster *et al.* 2004). Visitors that do not conform to the floral syndrome may also be pollinators (Waser *et al.* 1996; Johnson & Steiner 2000; Mayfield *et al.* 2001; Frank & Lounibos 2008; Ollerton *et al.* 2009). Such a situation was reported for bird-adapted mistletoe flowers pollinated by bees (Robertson *et al.* 2005). In this case, bees were considered as secondary or alternative pollinators. For some plant species, however, bimodal pollination systems have been described where two unrelated species or groups of pollen vectors, typically associated with different floral syndromes, act as pollinators of plant species that display flowers with intermediate pollination syndromes (Manning & Goldblatt 2005; see also Waser & Price 1990; Castellanos *et al.* 2003; Shuttleworth & Johnson 2008). A bimodal pollination system involving bees and long-tongued flies was found in two species, *Tritoniopsis toximontana* and *T. pulchella* (Iridaceae: Crocoideae), both having intermediate flower tube lengths and sugar concentrations, allowing both bees and flies to exploit the nectar. Another case, involving sunbirds and butterflies, was observed in *T. burchellii* and *T. triticea* (Manning & Goldblatt 2005).

The neotropical plant family Bromeliaceae comprises epiphytic or lithophytic forms, many with large showy flowers (Benzing 2000). Bromeliad flowers have floral traits adapted to pollination by birds (ornithophily), bats (chiropterophily) or insects ⁄ bees (entomophily ⁄ melittophily). There are more zoogamous than autogamous bromeliad species (Benzing 2000), and pollination by vertebrates dominates over insect pollination (Sazima *et al.* 1989). Bird pollination is the most frequent floral syndrome (Benzing 2000; Kessler & Krömer 2000), and hummingbirds appear to be effective pollinators (Benzing 2000). Usually, such conclusions are based on a record of flower visitors that are consistent with the floral syndrome and on observations on the fit in morphological features between flower and visitor (Kessler & Krömer 2000; Varassin & Sazima 2000; Canela & Sazima 2005). Sometimes these assumptions were strengthened by reports of the presence of pollen on visitors' bodies and contact with plant reproductive organs (Buzato *et al.* 2000; Canela & Sazima 2005). In the Atlantic rain forest of Brazil, bromeliads proved to be the most important plant family providing nectar for hummingbirds (Buzato *et al.* 2000; Cestari 2009). On the other hand, there are several reports of insect, e.g. bees and butterflies, visiting bromeliad flowers

(Bernardello *et al.* 1991; Wendt *et al.* 2001, 2002; Canela & Sazima 2003). In particular, many short-corolla bromeliads with otherwise typical ornithophilous syndrome are frequently visited by a high diversity of bees, suggesting the occurrence of bimodal pollination systems. Only experimental tests can reveal the pollination effectiveness of each visitor species (e.g. Keys *et al.* 1995; Freitas & Paxton 1998; Botes *et al.* 2009; Bumrungsri *et al.* 2009), but so far, pollination effectiveness of visitors to bird-adapted bromeliad flowers has not been tested.

Pollination success not only depends on the taxon but also on the motivation and behaviour of the visitor (Théry *et al.* 1998). Among floral rewards, nectar is the most important energy source for flower visitors (Krömer *et al.* 2008). In particular, hummingbirds are specialised in nectar feeding, so quality and quantity of nectar in flowers is an important selection feature for floral choice. There are several studies giving nectar volumes and concentrations for Bromeliaceae. Krömer *et al.* (2008) provided the most comprehensive report on nectar secretion patterns in 111 species of Bromeliaceae, with data on nectar concentration and sugar composition, but no volumes were recorded. Bernardello *et al.* (1991) analysed chemical nectar composition of 20 bromeliad species, finding hexose-rich nectars in two species of the subfamily Bromelioideae; these authors also did not relate composition to volume. Nectar composition examination in 18 species of bromeliads (Buzato *et al.* 2000) resulted in a mean sugar concentration of between 17.0% (*Vriesea morreniana*) and 38.9% (*Nidularium procerum*). Nectar volume ranged from 3.4 µl (*Tillandsia stricta*) to 54.1 µl (*Neoregelia johannis*) per flower. Canela & Sazima (2003) tested nectar volume and concentration at 2-h intervals for flowers of *Ae. pectinata*. They found that nectar production increased in the morning hours, with the highest volume at 08:00 h (median 21.5 µl), and highest concentration at 06:00 h (median 30%). This nectar secretion pattern was correlated to the frequency of hummingbird visits. According to Krömer *et al.* (2008), bromeliad nectar only contains sucrose and two hexoses, glucose and fructose, and the proportion of sucrose to these hexoses is associated with pollinator type rather than phylogenetic relationship. As yet, the diel pattern of nectar composition and the corresponding volume, both features strongly influencing the attractiveness of bromeliad flowers to pollinators, has not been determined.

Our study species, *Aechmea nudicaulis* (L.) Grisebach (Bromeliaceae: Bromelioideae), is widespread from Mexico to southern Brazil. The plant is common in the coastal areas of Santa Catarina (Reitz 1983). Plants form patches due to clonal growth of individual plants (Sampaio *et al.* 2002), and each ramet (i.e. rosette) can produce only one inflorescence during its lifetime. Little is known about its reproductive system, but the apparent floral traits, such as tubular shape, eye-catching combination of red bracts and yellow flowers, as well as nectar concentration and volume (mean concentration 27.2 ± 0.5%, mean volume of 14.5 ± 5.0 µl, Buzato *et al.* 2000) (Fig. 1A) suggest hummingbirds as most effective pollinators (Faegri & van der Pijl 1971; Sick 1993). In fact, hummingbirds have been reported as visitors to *Ae. nudicaulis* flowers in the Atlantic forest in São Paulo State, and are presumed to be the sole pollinators (Buzato *et al.* 2000; Machado & Semir 2006). As we found pollen of *Ae. nudicaulis* in larval provisions of the orchid bee *Euglossa annectans* (Cortopassi-Laurino *et al.* 2009), we made preliminary field observations that revealed flowers to be also visited by bees. This raised the question whether bees or other insects observed at these inflorescences (Varassin & Sazima 2000; Piacentini & Varassin 2007) might also act as pollen vectors of these plants. However, no detailed information about pollination biology of this species is as yet available.

As part of a project studying the species richness of bromeliad flower visitors and the diversity and nature of their animal–plant interactions, this study focused on revealing those visitors that maintain a beneficial relationship with the plants, in particular the pollinators. Specifically, we aimed to detect the capacity and, where present, to quantify the effectiveness of the visitor taxa in pollinating *Ae. nudicaulis* and to relate this to visitation frequency. To study the pollination system, it was essential to first analyse the floral phenology and reproductive system, as well as the quality and timing of floral rewards. Furthermore, we experimentally tested pollination effectiveness for some of the most frequent flower visitors.

MATERIAL AND METHODS

Study sites and study species

The study was conducted on Santa Catarina Island, southern Brazil, during four flowering seasons of *Aechmea nudicaulis*, from September to December, between 2005 and 2008. Field work was carried out at three sites: (i) a dune vegetation area (‘restinga’; Sampaio *et al.* 2002) between Joaquina Beach (27°37’37’’ S, 48°26’59’’ W) and Campeche Beach (27°40’38’’ S, 48°28’48’’ W); (ii) RPPN Morro das Aranhas (Private Reserve of Natural Heritage, 27°28’11’’ S, 48°23’04’’ W), also dune vegetation; and (iii) a hillside secondary forest area in Santo Antônio de Lisboa (Zillikens *et al.* 2001; 27°30’26’’ S, 48°30’28’’ W). These study sites were chosen to obtain sufficient numbers of flowering plants. Because no differences in plant biology and floral traits due to habitat influence between populations on Santa Catarina

Island were found, we pooled the data obtained from these sites. Time specification is given in standard time.

The bromeliad species *Ae. nudicaulis* occurs on Santa Catarina Island in natural habitats and in urban areas on trees and also on the ground. The hermaphrodite flowers of *Ae. nudicaulis* have no noticeable odour. Anthers are located inside the corolla and the stigma is situated beneath the anthers (Fig. 1C). Flowers mainly open between 05:00 h and 18:00 h (see Figure S1) and wither thereafter. Fruits are first green (Fig. 1D), then bright orange (Fig. 1B) and develop within 4 weeks. The somewhat sticky pollen grains (Fig. 1E) are oval and biporate, one grain measures 36 x 30 µm (Zillikens *et al.* 2008).

Flowering phenology and reproductive system

To assess the flowering phenology of *Ae. nudicaulis*, flowering plants were counted in the field every week (for detailed method see Figure S2). The total number of flowers per inflorescence was counted for 54 inflorescences (plants from Santo Antônio, Campeche and Joaquina) and the number of open flowers per day was recorded for 17 inflorescences (plants from Santo Antônio) throughout the whole flowering phase. Additionally, the process of flower opening was monitored (see Figure S1).

Ovule number was counted for 151 flowers [n = 20 plants from Santo Antônio, mean 6.86 ± 6.88 (SD) flowers / plant]. Anther dehiscence was observed during field observations in Santo Antônio and in the laboratory. The period when pollinators can facilitate the production of seeds was assessed in the laboratory. For this experiment, three plants from Santo Antônio were used to perform manual cross-pollinations (five flowers every hour between 05:00 h and 17:00 h, with 27, 23 and 15 flowers per plant). Care was taken that the chosen plants did not belong to the same clonal patch. Seed set was determined by counting seeds in the developing fruits. The breeding system was examined with hand-pollination treatments on plants from Santo Antônio that were taken to the laboratory: autonomous self-pollination (4 + 3 + 3 flowers, three plants), manual self-pollination (5 + 5 flowers, two plants) and manual cross-pollination (6 + 4 flowers, two plants) (Dafni 1992). In addition, flowers in the field [Campeche in 2008: n = 28 flowers of six plants, mean 4.7 ± 0.5 (SD) flowers / plant] were marked as controls for natural pollination. After 4 weeks, fruit and seed set were determined. The index of self-incompatibility (ISI) was calculated for this bromeliad species (Zapata & Arroyo 1978).

Nectar volume and composition

To collect nectar samples, plants from Santo Antônio were taken to the laboratory. This was done to exclude floral visitors without the need to bag inflorescences, which might have changed the nectar composition, as suggested in Wyatt *et al.* (1992). Nectar was collected from two, six and eight flowers of three plants. Six samples per flower were collected from 05:00 h to 15:00 h (at 2-h intervals, n = 96 samples) with 2-µl microcapillaries (end-to-end capillaries, Hirschmann Laborgeräte GmbH, Germany) and stored in Eppendorf cups containing 1 ml 70% ethanol (HPLC grade, Merck, Germany). The sample content (n = 96, six samples per flower) of sucrose, fructose and glucose was analysed using HPLC (Piechowski 2007). Total volume was measured by removing the remaining nectar with 5- to 20-µl micropipettes (Assistent, Germany), and adding to this volume the previously collected 2 µl. Total energetic value of the nectar was determined by multiplying total sugar weight (µg) for glucose, fructose or sucrose by 0.0156 $J*\mu g^{-1}$, 0.0157 $J*\mu g^{-1}$ or 0.0165 $J*\mu g^{-1}$, respectively, and summing the products. These energy-per-mass factors were calculated using the per mol energetic equivalents of the three sugars (Wieser 1986). The nectar measurements at 05:00 h were the first possible (immediately after flower opening) and, thus, differ from the other measurements in the sense that we do not know the length of the period that nectar was present in the still-closed buds. Sugar concentration was not measured after 15:00 h because almost no nectar was present at that time. For data analysis, we calculated means of sugar concentration and nectar volume for each plant, resulting in three values per time of day (hence, n = 18).

Effectiveness of flower visitors for seed production

To determine visitor frequencies for each visitor species, field observations were conducted at all sites, on both terrestrial (n = 72) and epiphytic (n = 4) plants, but were limited to a height of 5 m above ground because of restricted visibility (a total of 285 observation hours). Observations of inflorescences were performed between 05:00 h and 17:00 h (almost the whole period of anthesis, Figure S1), continuous observation sessions on inflorescences lasted between 1 and 8 h (observations started between 05:00 h and 14:00 h). Relative visitation frequencies were calculated by expressing the number of individual visits of each visitor taxon as a percentage of the total number of visits (n = 1359).

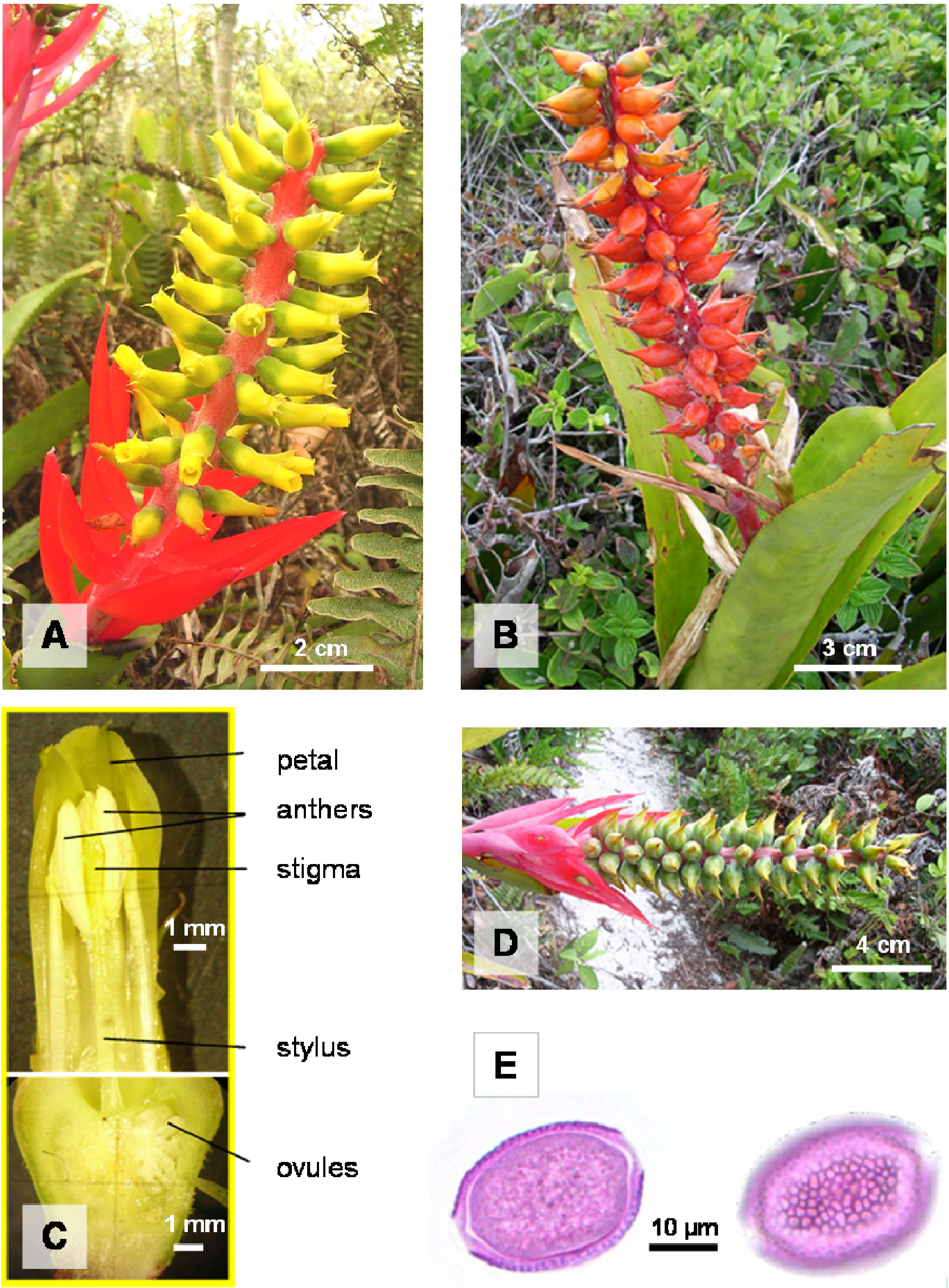

Fig. 1. Floral traits in *Aechmea nudicaulis* in Florianópolis on Santa Catarina Island, Brazil. **A**: inflorescence with open flowers. **B**: ripe fruits. **C**: longitudinal section of open flower. **D**: unripe fruits. **E**: pollen grains (Zillikens *et al.* 2008).

Since we defined individual pollinator effectiveness to be related to the number of seeds resulting from a single visit (in agreement with Motten *et al.* 1981; Schemske & Horvitz 1984), we carried out pollination experiments (only terrestrial plants). The tests were carried out under field conditions in 2006 and 2008 in Santo Antônio and Campeche. Among the flower visitor species, we chose those that appeared to be more frequent and capable of pollination because they seemed to contact the reproductive organs: two bird, five bee and one butterfly species. The bee species covered a wide range of size, from small halictid sweat bees (Halictidae: Augochlorini) to very large bumble and carpenter bees (Apidae: Bombini, Xylocopini, respectively). *Heliconius ethilla* (Lepidoptera: Nymphalidae: Heliconiinae) was selected as a representative of all butterfly species recorded as flower visitors. For other visitor species, e.g. small stingless bees (Apidae: Meliponini) or castniid butterflies, we could not obtain any data because they did not visit the flowers during the pollination tests. To allow only a single visit per visitor and flower, whole inflorescences were bagged the day before anthesis and unbagged on the next morning at the start of the experiment. If a visitor did not approach all open flowers, only the visited flowers were covered with Eppendorf cups to prevent further contact while waiting for the remaining flowers to be visited. When all flowers had been visited once, the inflorescence was bagged again. After fruit ripening, the developed seeds were counted.

Because a census in 2008 revealed a high variance of total ovule number per flower (see Figure S3), the number of seeds was expressed as a ratio to ovule number for each fruit, thus relating pollination success to potential maximum seed set. The seed numbers obtained in 2006 were expressed as a percentage of the mean ovule number. As a positive control, 32 flowers of seven inflorescences were marked on a sunny day in Campeche in 2008 and left unbagged. The seed⁄ovule ratios obtained from single visits were used as a measure for the pollination power of individual visitors. The value of these visitors for the plant, however, also depends on the average frequency with which they visit the flowers (Schemske & Horvitz 1984). Thus, to evaluate visitor taxa in this respect, we weighted – i.e. multiplied – the seed⁄ovule percentages of each visit by the relative visitation frequency of the visitor's taxon. The per taxon means of the products obtained were then used as an indicator of pollination effectiveness. For better comparison, we standardised these means so that the highest value (that of the hummingbird, *Thalurania glaucopis*) is 1, and all other values reflect the relative effectiveness compared with this taxon.

Statistical analysis

To test whether the pollination treatment influenced seed number, we used analysis of variance by ranks (Kruskal–Wallis test) following Zar (1999). *A posteriori* nonparametric Tukey-type multiple comparisons (Zar 1999) were performed among all pairs of treatments using the Q test statistic (Zar 1999; equation 11.27) and a standard error accounting for considering tied ranks (Zar 1999, equation 11.28).

We performed paired *t*-tests to compare sugar concentrations, and linear Pearson correlations to uncover relationships among sugar concentrations and between nectar characteristics (sugar concentrations and nectar volume) and time of day. The *t*-tests and correlations were calculated using Microsoft Excel 2003. When multiple tests were conducted with the same dataset, we corrected the P-values by multiplication by the number of tests, conforming to the unweighted Bonferroni method (Shaffer 1995). As a result, the presented P-values can be compared to the usual levels of significance (*e.g.*: a P-value of 0.0001 obtained from one of nine multiple tests is presented as $P = 0.0009$). When the correction resulted in a value ≥ 1, $P = 0.9999$.

Since our study question was whether bees can be a reasonable alternative to hummingbirds as pollinators, we compared the individual (relative frequency x seed⁄ovule ratio) values between T. *glaucopis* and the pooled bee taxa with a Mann–Whitney *U*-test using the statistical software package JMP 8.01 (SAS Institute Inc., 2009). All P-values were derived from two-tailed tests.

RESULTS

Floral biology and reproductive system

The mean number of flowers per inflorescence was 47.4 ± 16.2 SD (range 16–118, n = 54 inflorescences). One to 15 flowers opened per day (mean = 5; n = 17 inflorescences), with a peak on the fourth day of flowering (maximum of 9 days with open flowers). The mean number of ovules per flower was 198.5 ± 64.2 (SD, n = 151 flowers, 20 inflorescences). Anther dehiscence occurred simultaneously with initiation of nectar production. Initiation of seed ripening was possible throughout the whole of anthesis (Figure S4).

The pollination treatment had a significant influence on seed number ($H = 37.87$; $df = 3$; $P < 0.001$). *A posteriori* comparisons revealed that the self-pollination treatments were not significantly different from each other ($P > 0.5$); the same was true for cross- and natural pollinations (Fig. 2). However, both self-pollination treatments differed from the other two treatments at a 0.001 significance level. *Aechmea nudicaulis* had a self-incompatibility index (ISI) of 0, indicating complete self-incompatibility since no fruits developed after self-pollination.

Floral rewards

Floral nectar is an important reward for flower-visiting birds and insects. Nectar volumes and sugar concentrations were high in the morning. The mean nectar volume produced per hour was 2.4 µl (range 1.5–3.5 µl/h^{-1}); the volume significantly decreased throughout the day (linear correlation; r = -0.5509, P = 0.0178, n = 18). The daily total volume per flower was 31.84 ± 6.62 µl (mean ± SD, n = 16 flowers). Mean sugar concentration was 23.2 ± 1.94% (weight/weight = w/w ± SD). The nectar was mostly sucrose-dominant, with a mean sucrose proportion between 45.3% (at 13:00 h) and 74.1% (at 07:00 h) and an overall mean sucrose/hexose ratio of 2.4. Sucrose concentration was high in the first hours of nectar secretion (mean at 05:00 h: 262 µg µl^{-1}, Fig. 3) and decreased significantly in the following hours, while the concentration of hexoses (mean 43.7 µg µl^{-1}; Fig. 3) remained nearly constant throughout the day (Table 1). Sucrose concentration was significantly higher (Table 1) than that of both fructose (mean difference =124.18 µg µl^{-1}) and glucose (mean difference = 130.48 µg µl^{-1}). Fructose concentration was significantly higher than that of glucose (Table 1; mean difference = 6.30 µg µl^{-1}). The total sugar secretion per flower was 8631.2 ± 1978.2 µg sugar d^{-1} (mean ± SD, n = 16 flowers). The mean energy value per flower was 140.1 ± 32.3 J (± SD, n = 14). Multiplied by an average of 47.4 flowers per inflorescence, this results in a total of 412 mg sugar secreted and an energy value of 6.68 kJ per flowering plant. Pollen, another important reward for many visitors, especially bees, was usually removed from the anthers within the first 2 h of visitor activity.

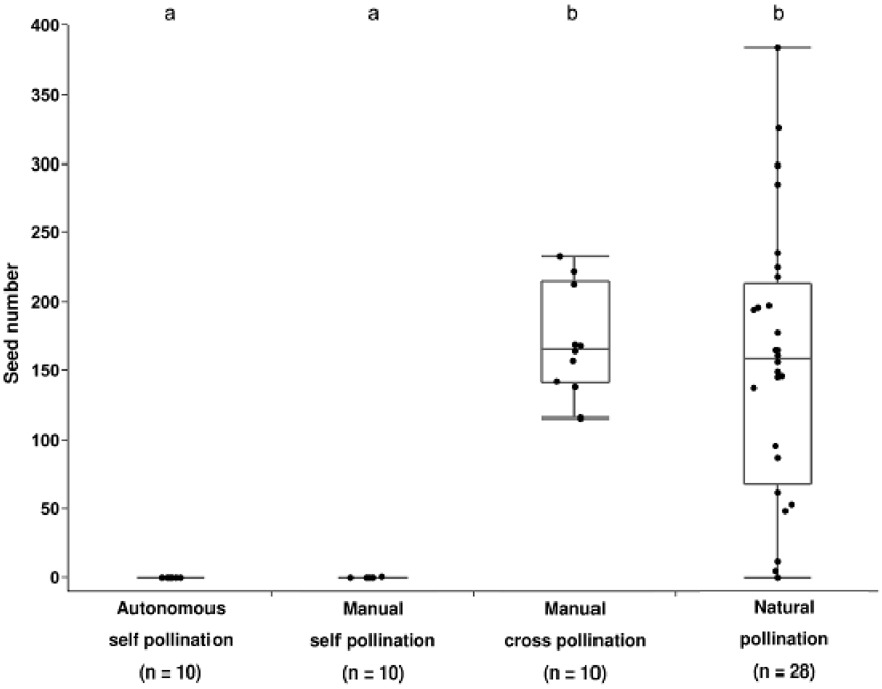

Fig. 2. Seed number of fruits of *Aechmea nudicaulis* after different treatments. Points were jittered for better visibility. Box plots show median (middle line) and inter-quartile range (box); the whiskers give the range of all points that fall within the following limits: upper quartile +1.5 x (interquartile range), lower quartile -1.5 x (interquartile range). The letters above the plots indicate whether treatments were significantly different (different letters, $P < 0.001$) or not (same letters, $P > 0.5$).

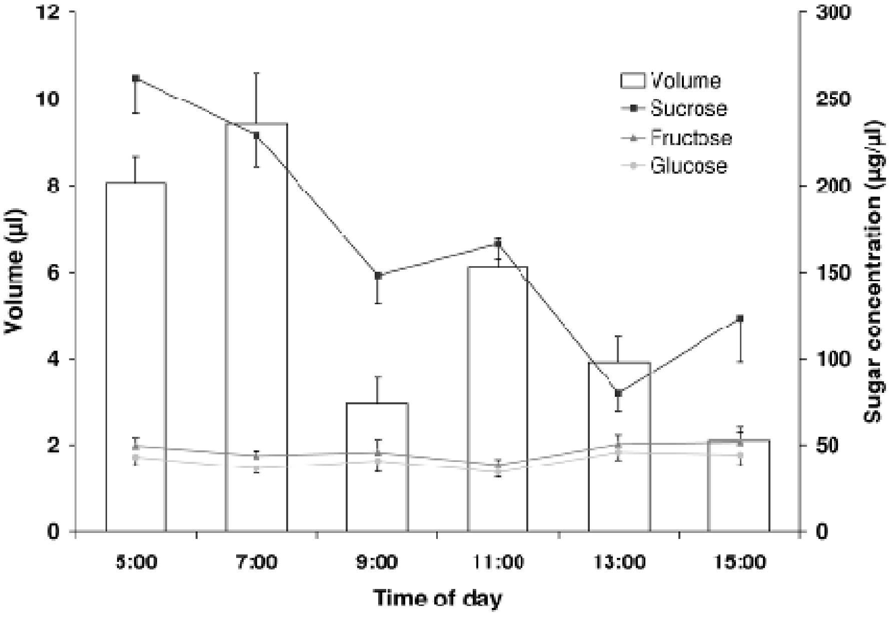

Fig. 3. Diurnal patterns of volume and concentration of the main sugars sucrose, glucose and fructose per flower (means ± SEM, n = 3 plants) in nectar secreted during a 2-h period from flowers of *Aechmea nudicaulis*. For data measured at 05:00 h, the period of nectar production is not precise.

Effectiveness of bird and insect pollinators

In terms of seed⁄ovule ratio, the pollination experiments revealed *Thalurania glaucopis* as the most effective pollinator in both years, followed by augochlorine sweat bees (Halictidae), xylocopine bees (Apidae), the bumble bee *Bombus brasiliensis* (Apidae) and the butterfly *Heliconius ethilla* (Nymphalidae) as alternative pollinators, although these were less efficient than the hummingbird. Small carpenter bees of the genus *Ceratina* (Apidae) and the orchid bee *Euglossa annectans* (Apidae), as well as the passerine bird *Coereba flaveola*, the Bananaquit (Passeriformes: Thraupidae), pollinated a few ovules (Table 2). Visitation frequencies of the bee taxa *Xylocopa, Augochlorini* and *B. brasiliensis* exceeded that of the rarely visiting hummingbird T. *glaucopis*. Hence, visitation was dominated by bees (Table 3), and the resulting effectiveness ranking, calculated from frequency and seed set, produced values between 0.19 and 0.57 for these bees (Table 3). Even when we considered relative visitation frequencies, T. *glaucopis* still had the highest relative pollination effectiveness and was significantly more effective than all bees together (Mann–Whitney *U*-test; $\chi^2 = 19.89$; df = 1; $P < 0.0001$), reaching 85% of hummingbird effectiveness.

DISCUSSION

Flowering phenology and reproductive success

Although individual *Ae. nudicaulis* plants flower over a short period and produce only one inflorescence, the flowering of the whole population extends over several months (‘steady state’, Gentry 1974; Figure S2); similar phenology occurs in *Ae. lindenii* (E. Morren) Baker (Dorneles 2006; Lenzi *et al.* 2006). The pattern of individual flowering occurring over a short period seems to be less common in bromeliads than the ‘steady state’ pattern (Benzing 2000).

Our pollination experiments revealed that flowers of *Ae. nudicaulis* do not produce seeds through self-pollination and are therefore completely self-sterile. As species of *Aechmea* can produce large patches of clonal plants by sprouting from ramets (Sampaio *et al.* 2002), admitting self-pollination would impose a high risk of inbreeding, even for pollen transferred between different inflorescences. Thus, xenogamy can be regarded as a strategy that is well adapted to ensure out-crossing in these bromeliads. Under natural conditions on Santa Catarina Island, pollination success was high in both secondary forest and dune vegetation (Schmid, personal observation), indicating that reproduction is secure, independent of vegetation type. This implies that animal pollen vectors of *Ae. nudicaulis* occur in a wide range of environments.

Table 1. Statistical tests used to assess sugar concentration. Concentrations of three sugar types were linearly Pearson-correlated with time and with each other. Paired *t*-tests were used to evaluate differences between each pair of sugar types. n = 18 for all tests; df = 1 for all correlations; df = 17 for all *t*-tests. P-values were Bonferroni-corrected by multiplication by 9; when this correction yielded value was ≥ 1, P = 0.9999.

	Correlation	
Comparison	r	P
Sucrose-time	-0.8322	0.0002
Fructose-time	0.2310	0.9999
Glucose-time	0.3057	0.9999
Fructose-Glucose	0.9681	< 0.0001
Sucrose-Glucose	-0.5882	0.0922
Sucrose-Fructose	-0.4930	0.3389
	Paired t tests	
	t	P
Sucrose-Glucose	7.3251	< 0.0001
Sucrose-Fructose	7.0340	< 0.0001
Fructose-Glucose	11.5077	< 0.0001

Nectar composition is adapted to hummingbirds

High concentrations and volumes of nectar in the early daytime hours are common in plants pollinated by hummingbirds (Baker 1975; Feinsinger 1976; Lotz & Schondube 2006; Johnson & Nicolson 2008). Furthermore, relatively dilute but sucrose-rich nectar (sucrose content approx. 40–60% of total sugars; Johnson & Nicolson 2008) is a common feature in hummingbird-pollinated flowers (Baker 1975; Heinrich 1975; Freeman *et al.* 1984; Stiles & Freeman 1993; Krömer *et al.* 2008) with sugar concentrations of 15–25% (w / w; Roberts 1996; Johnson & Nicolson 2008). This seems to be an adaptation to hummingbird visitation because this concentration meets the optimal sugar intake and digestive efficiency for hummingbirds (Lotz & Schondube 2006); therefore, in *Ae. nudicaulis*, nectar presentation and composition are evidently adapted to attract hummingbirds. However, Bernardello *et al.* (1991) reported that *Ae. distichantha* Lemaire has hexose-rich nectar and is also pollinated by hummingbirds (Benzing 2000), while *Ae. bracteata* (Swartz) Grisebach secretes sucrose-rich nectar (Percival 1961) but is spontaneously autogamous (Benzing 2000). Therefore, secure assumptions about pollination mode cannot be made based on sugar composition alone.

The diurnal pattern of nectar volume and concentration in *Ae. nudicaulis* indicates higher attractiveness of flowers in the morning. Similar patterns were found in *Ae. organensis* Wawra, *Ae. ornata* Baker (Machado & Semir 2006) and *Ae. pectinata* Baker (Canela & Sazima 2005) in São Paulo State, and in *Ae. beeriana* L.B. Sm. & M.A. Spencer in Manaus (Nara & Webber 2002). Herrera *et al.* (2008) provided evidence that yeast communities in nectar of three plant species (*Helleborus foetidus, Aquilegia vulgaris*, and *Aquilegia pyrenaica cazorlensis*) had an impact on nectar by breaking down sucrose and digesting glucose so that fructose accumulated. The nectar composition pattern found by these authors resembled that of *Ae. nudicaulis* in terms of sucrose reduction throughout the day. However, in contrast to Herrera *et al.* (2008), fructose concentration did not change. The flowers used in our experiment were probed in the laboratory, therefore all visitors were excluded. Since yeasts were only found in visited flowers (Canto *et al.* 2007; Herrera *et al.* 2008), contamination of our flowers is highly improbable, although we did not examine flowers for yeasts. Consequently, the sugar composition determined in *Ae. nudicaulis* nectar was not altered by yeasts and reflects the real pattern of sugar secretion, but not necessarily the actual condition of nectar concentrations found in situ at a given point during anthesis.

Calculation of the energy demand in the hummingbird *Selasphorus flammula* revealed that this bird would need to visit 313 *Salvia* flowers to obtain enough sugar to fuel its daily activities (Hainsworth & Wolf 1972). Similarly, *Thalurania glaucopis*, identified by us as the main pollinator of *Ae. nudicaulis*, with a mean body mass of 5 g (Marini *et al.* 1997), has an energy demand of 4.19 kJ/h^{-1} (1 kcal/h^{-1} in Poley 1994). This results in an energy uptake demand of 50 kJ/d^{-1}, which is consistent with a daily energy value of sugar secreted by 355 *Ae. nudicaulis* flowers. Thus, assuming that no other nectarivorous competitors were present, *T. glaucopis* could meet their energy demand from an equivalent of 71 inflorescence visitations (a mean of five open flowers per day) every 2 h only from these plants. Thus it appears feasible that a hummingbird could meet its daily energy demand by only visiting *Ae. nudicaulis* flowers.

Table 2. Effectiveness of pollination by flower visitors to *Aechmea nudicaulis* on Santa Catarina Island, Brazil. Values are given as percentage of seed development = pollinated ovules ⁄ total ovules. In 2006, seed number was set in relation to mean ovule number, whereas in 2008 the seed and ovule numbers were determined for every fruit.

	Seed set 2006 + 2008					Seed set 2006		Seed set 2008	
Pollinator	N	%	% (range)	No. seeds /fruit (mean)	(range)	%	No. seeds (mean)	%	No. seeds (mean)
Natural pollination	32	68.1	0-99.5	156.5	0-385	-	-	68.1	156.5
Thalurania glaucopis	60	22.8	0-98.9	35.3	0-177	10.3	20.0	64.1	104
Coereba flaveola	4	0.1	0-0.5	0.3	0-1	0.1	0.3	-	-
Heliconius ethilla	3	3.0	0-7.9	3.7	0-10	-	-	2.8	3.7
Bombus brasiliensis	30	1.8	0-11.6	3.5	0-23	1.8	3.5	-	-
Euglossa annectans	79	0.4	0-6.1	0.6	0-11	0.3	0.6	0.4	0.6
Xylocopa sp.	46	3.3	0-53.9	6.4	0-107	5.1	6.5	1.2	2.0
Ceratina sp.	9	1.2	0-3	2.4	0-6	1.2	2.4	-	-
Augochlorini	29	2.2	0-46.3	3.1	0-63	0.1	0.1	6.1	8.9

N: number of flowers treated; %: mean percentage of fertilised ovules per fruit.

Pollination ecology: influence of time of day and season

In *Ae. nudicaulis*, pollination is possible during the whole period of anthesis. On warm and sunny days when flower visitors become active at dawn, pollination will likely occur in the morning because pollen is usually exhausted by noon. However, if unfavourable weather conditions, such as heavy rains, strong winds or low temperatures in the morning, impede or reduce the activity of flower visitors and delay removal of pollen, successful pollination is still possible in the afternoon (Figure S4). Flower visitation is, however, unlikely to occur after 17:00 h, when nectar is no longer measurable. Even in the first case, continued production of nectar likely attracts and binds the visitors to the inflorescences. By revisiting them repeatedly, visitors (e.g. hummingbirds or large bee species) are more likely to learn the colour pattern and the position of flowering plants. This is an important prerequisite for establishing a fixed foraging trapline. Such behaviour favours successful pollination in *Aechmea* because simultaneously flowering bromeliads are scattered rather than clumped. Trapline behaviour is known to be performed by hummingbirds as well as bumblebees (Janzen 1971; Feinsinger 1976; Thomson 1996), thus enhancing successful pollination (Ohashi & Thomson 2009).

Pollination efficacy in *Ae. nudicaulis* may decrease with increasing number of inflorescences per clonal patch due to the higher occurrence of inbreeding in a local plant community. A higher seed set under natural conditions, as observed in 2008, could be the result of low nectar standing crop for hummingbirds in that spring. Due to a long period of heavy rainfall, there were fewer flowering plants within a clonal patch than in previous years, so pollen transfer between unrelated individuals might have occurred more often and resulted in higher reproductive success.

Table 3. Relative efficiencies of two birds, one butterfly and five bee species (and all bees pooled together) in pollinating flowers of *Aechmea nudicaulis*. Effectiveness values were calculated by multiplying each species' relative visitation frequency by the mean seed ⁄ ovule ratio resulting from single visits. This product was divided by the value of *Thalurania glaucopis*, thus facilitating a comparison with this hummingbird species.

flower visitor	relative visitation frequency (%)[a]	mean seed/ovule ratio (%)	frequency x mean seed/ovule ratio	relative effectiveness
Thalurania glaucopis	6.15	22.83	140.31	1
Coereba flaveola	0.60	0.13	0.08	0.00
Heliconius ethilla	0.15	2.98	0.45	0.00
Augochlorini	12.14	2.15	26.15	0.19
Bombus brasiliensis	44.23	1.81	80.21	0.57
Ceratina sp.	0.52	1.23	0.65	0.00
Euglossa annectans	5.25	0.35	1.84	0.01
Xylocopa sp.	12.89	3.25	41.85	0.30
Bees pooled	75.19	1.58	118.80	0.85

[a] Percentages do not sum up to 100% because not all visitors recorded were tested for efficiency.

Does the pollination syndrome match the actual pollinators?

Most floral traits of *Ae. nudicaulis* are consistent with the ornithophilous floral syndrome. The flowers have tubular corollas made of robust petals, brightly coloured flowers and bracts, no landing platform, high secretion of sucrose-dominant nectar with a low total sugar content, absence of scent and immediate presentation of all pollen, indicating short-billed hummingbirds as the best-adapted pollinators of *Ae. nudicaulis* (Varassin & Sazima 2000). In fact, our pollinator effectiveness experiments revealed that *T. glaucopis* is the most efficient pollen vector for seed set after a single visit. However, some floral traits lie between the ornithophilous and melittophilous syndrome, such as yellow petals, anthers located inside the flowers but close to the corolla opening, and a relatively short corolla (effective length 12.3 ± 0.5 mm, Buzato *et al.* 2000; see also Fig. 1C). In addition, we found an intermediate mean sucrose proportion that was higher in the first hours of nectar secretion, was more attractive to hummingbirds, but decreased later in the day and then was better adapted to bees and butterflies (Krömer *et al.* 2008). In particular, corolla length, anther location and sugar composition make the flowers attractive for bees that collect both nectar and pollen. The rather short corolla also allows access to floral rewards by the nectarivorous perching bird *Coereba flaveola* and the butterfly *Heliconius ethilla*. In our pollination experiments, the large bumble and carpenter bees as well as the small augochlorine sweat bees actually induced seed set, albeit with much lower effectiveness than the hummingbirds. Similarly low contributions to seed set by bees in ornithophilous *Aloe* flowers in South Africa were observed by Botes *et al.* (2009). This is because bees, in contrast to hummingbirds, groom their bodies frequently so that the pollen is stowed in the scopae and is no longer available to pollinate flowers (Thomson 1986; Schmid personal observation). Castellanos *et al.* (2003) found a more rapid decline in the pollen carryover by bumble bees than by hummingbirds on *Penstemon strictus* flowers and attributed this difference to the frequent grooming behaviour of the bees. Similar to bees, most of the pollen collected by heliconiine butterflies at the base of their proboscis is lost for pollination because they rapidly digest it (Gilbert 1972; Boggs *et al.* 1981; O'Brien *et al.* 2003) so that few loose grains remain viable. *Coereba flaveola* was reported by Sazima & Sazima (1999) to be a pollinator of three bromeliads (*Ae. bromeliifolia, Ae. distichantha* and *Acanthostachy strobilacea*) in São Paulo State, Brazil, but the conclusion of these authors was only based on observing the birds feeding at the flowers. In our experiments, *C. flaveola* completely failed to promote seed set. Thus, the visitor species *C. flaveola* and *H. ethilla* can be considered flower parasites, acting as robbers of nectar or pollen. However, the perception of low effectiveness of bees in pollinating *Ae. nudicaulis* flowers changes if visitation frequency is taken into account. Calculating relative effective values showed that, besides the hummingbird, *Xylocopa, Bombus* and some species of augochlorine bees should also be considered functional pollinators. The relatively poor effectiveness of these bees may be compensated by their much higher frequency of flower visitation. Taking all the bee species, they visited flowers over 10-times more often than hummingbirds, resulting in a pollination effectiveness almost as high as that of *T. glaucopis*. Therefore, on Santa Catarina Island, the pollination system of *Ae. nudicaulis* can be considered bimodal, using species from two different pollinator types as pollen vectors. Manning & Goldblatt (2005) considered bimodal pollinator systems to be instable. They argued that if one effective pollinator becomes less frequent, the system would tend to move towards the other pollinator type. Aigner (2001), however, suggested that intermediate flower syndromes are not necessarily transitional steps between highly specialised systems, but may be optimal under specific circumstances (pollinator environment) at a given locality. Making use of different pollinator groups constitutes a redundancy in pollination services, which may be one reason allowing *Ae. nudicaulis* to occur within a wide geographical range and to flourish in different vegetation types with varying microhabitats.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1. Time scale of flower development in *Aechmea nudicaulis*. The flowering process was monitored by observing the growth of 54 flowers (six plants from Santo Antônio de Lisboa) every hour between 15:00 and 09:00 h on three subsequent nights in the laboratory. Between 09:00 and 15:00 h there was no change in flower development; hence during this period the buds were not systematically examined. Bars display the length of each developmental phase. The whole flowering process, from the beginning of petal expansion to flower closure, took about 60 h.

Figure S2. Flowering period of *Aechmea nudicaulis* at three locations (Santo Antônio de Lisboa [red line], Campeche [green line], and Joaquina [blue dashed line]) on Santa Catarina Island (2007-2008) as observed by weekly counts along defined trails. Since sample size differed between sites the number of inflorescences with at least one open flower is given in % of the maximal weekly number of flowering plants. The flowering period of *Ae. nudicaulis* extended from September to December whereas infructescences were present until mid February.

Figure S3. The relationship between ovule number and flower position on the inflorescence (flower height) was determined for 17 flowers of six plants. Flower height was recorded ordinally: (1) = low (at infl. base), (2) = middle, and (3) = high (at infl. top), and Spearman-correlated (JMP 7.02) with the total number of ovules. The number of ovules per flower was significantly negatively correlated with the position of the flowers (Spearman $\rho = -0.7722$; $P = 0.0003$; $n = 17$ flowers, 6 plants) with the basal flowers having highest ovule numbers.

Figure S4. Diurnal pattern of pollination success in *Aechmea nudicaulis* changes along the day. It is expressed as the number of seeds produced per fruit in *Ae. nudicaulis* after manual cross pollination conducted with three inflorescences every hour from 05:00 to 17:00 h ($n = 65$ flowers). Black line: linear regression ($r2 = 0.30$; $P < 0.0001$).

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Supplementary Figures:

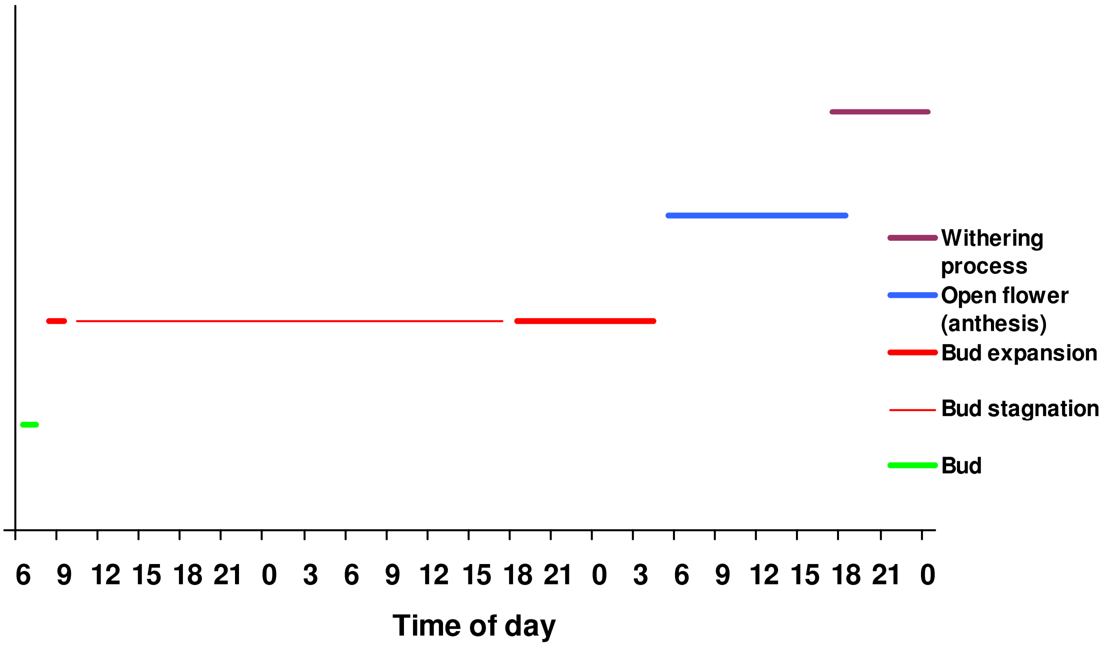

Fig. S1.

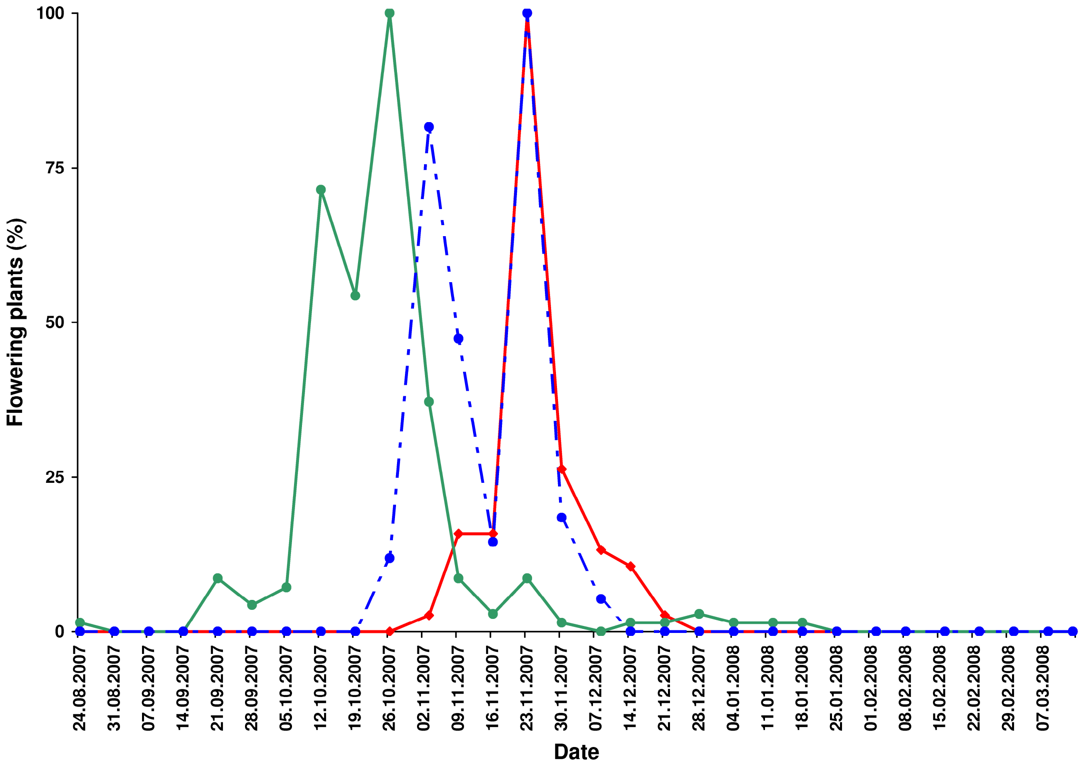

Fig. S2.

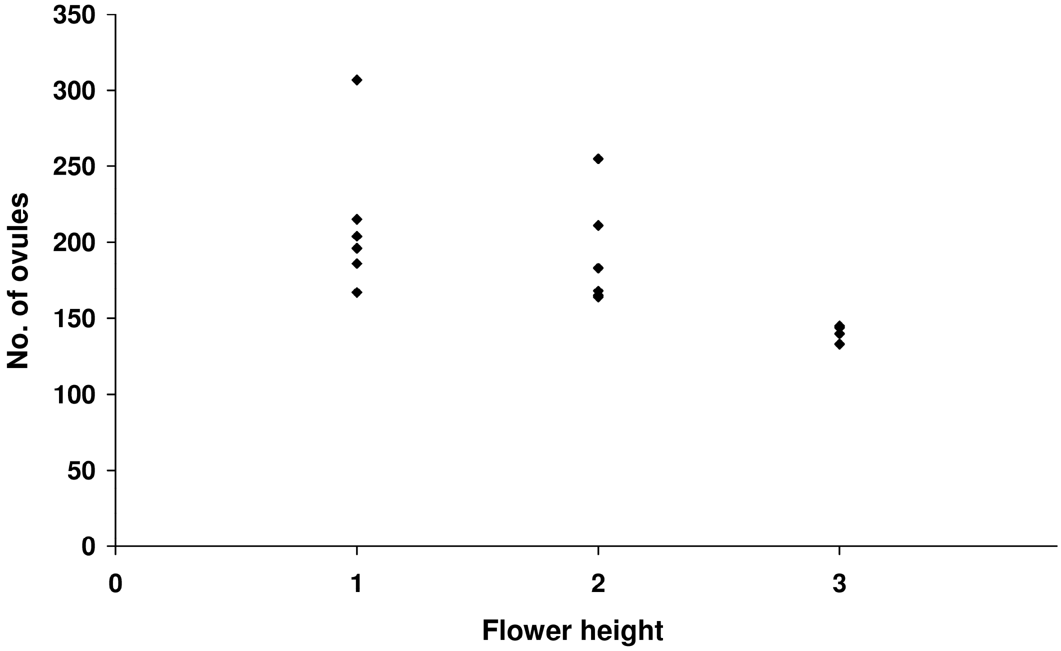
No. of ovules
350
300
250
200
150
100
50
0
0
1
2
3
Flower height

Fig. S3.

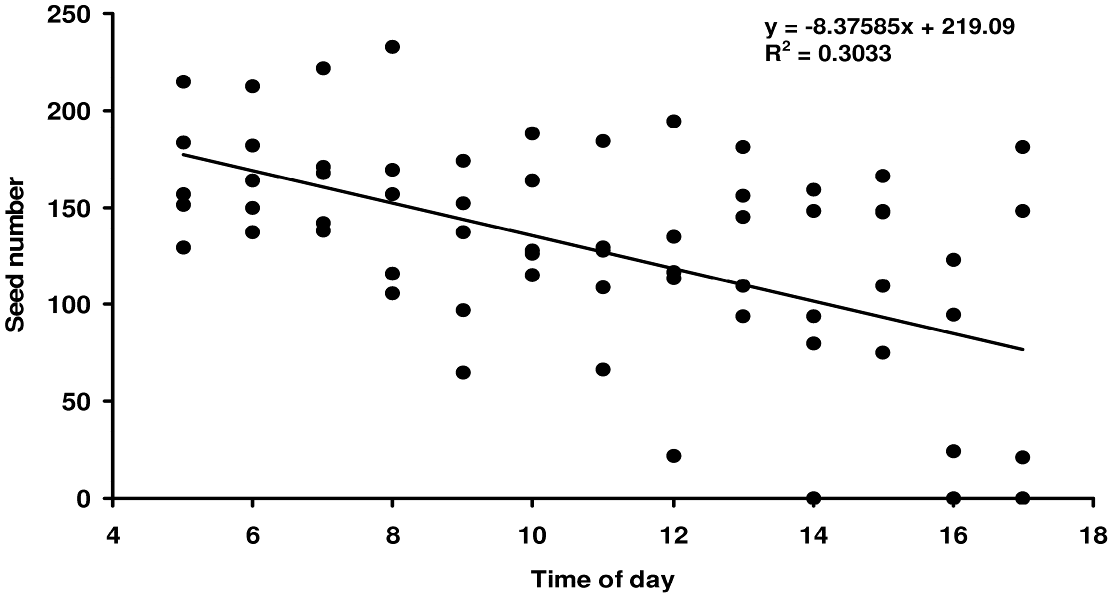
y = -8.37585x + 219.09
R² = 0.3033
Seed number
250
200
150
100
50
0
4
6
8
10
12
14
16
18
Time of day

Fig. S4.

Veröffentlichung 5: Schmid S, *Kamke R, Steiner J & A Zillikens (2010) Flower visitors of Aechmea nudicaulis (Bromeliaceae): Species richness, diversity, and visitation frequency in different habitats. In Revision bei Studies on Neotropical Fauna & Environment.*

Von *Aechmea nudicaulis* (Bromeliaceae) wurde das gesamte Blütenbesucherspektrum aufgenommen. Im Einzelnen wurden das Sammelverhalten der einzelnen Besucher, der zeitliche Verlauf der Blütenbesuche, sowie Beziehungen zwischen Bromelien und Tieren als auch zwischen den einzelnen Besuchergruppen erfasst. Die Besucherspektren von Sekundärwald und Restinga in SC und PR wurden verglichen, da bisher nur wenig über den Einfluss von Habitat und Breitengrad auf das Besucherspektrum von Bromelienblüten bekannt ist.

An den Blüten von *Ae. nudicaulis* wurden 40 Blütenbesuchertaxa registriert; die höchste Artenzahl im Sekundärwald in Paraná. Die Restinga auf der Insel von SC war am artenärmsten und auch die Besucherfrequenz war hier am niedrigsten. Die häufigsten Blütenbesuchergruppen waren Vögel, Bienen und Schmetterlinge. Die Artenzahlen der Besucher in den beiden Waldgebieten sowie der Restingagebiete waren untereinander sehr ähnlich, die Gebiete unterschieden sich aber in der Artenzusammensetzung. Der größte Unterschied im Artenspektrum bestand zwischen den beiden Waldgebieten in SC und PR. Während die Blüten in Paraná hauptsächlich von Kolibris besucht wurden (54,6% aller registrierten Besuche), dominierten in SC die Bienen (44,5 – 59,7% aller Besuche).

Obwohl sich kein signifikanter Zusammenhang zwischen Besucherfrequenzen und Uhrzeit nachweisen ließ, konnte eine tendenziell höhere Aktivität der Besucher in den ersten Stunden der Anthese am Morgen gezeigt werden. Dieses zeitliche Muster war in allen Untersuchungsgebieten ähnlich. Dabei hatten das Habitat und die geographische Lage einen Einfluss auf das Artenspektrum. Wir konnten nachweisen, dass die Blüten ein wesentlich breiteres Spektrum an Tieren anziehen als aufgrund ihrer Morphologie (Vogelblüten) zu erwartet war.

Unsere Studie zeigte außerdem, welchen Nutzen die Bromelien für den einzelnen Besucher hatten. Die Kolibris decken durch den Nektar ihren Bedarf an Energie. Bienen sammeln Pollen für ihre Brut und Nektar für ihren eigenen Bedarf oder um ihn dem Pollen unterzumischen. Prädatoren wie Spinnen, Wanzen und Gottesanbeterinnen machen Jagd auf potentielle Bestäuber. Herbivore Wanzen und Käfer ernähren sich vom Blütengewebe selbst. Ameisen, kleine Bienen und Wespen sammeln vorwiegend extrafloralen Nektar, da dieser höher konzentriert ist als der florale Nektar und außerhalb der Blüte leichter zugänglich ist.

Generalistische Herbivoren wie Käfer, Kakerlaken und Heuschrecken sind unspezifisch mit den Infloreszenzen assoziiert, während Wespen der Gattung *Eurytoma* und Bläulingsraupen der Gattung *Strymon* spezifische Beziehungen zu den Blüten- beziehungsweise Fruchtständen haben.

Veröffentlichung 5: Flower visitors of *Aechmea nudicaulis* (Bromeliaceae): Species richness, visitation frequency, and interactions in different habitats of southern Brazil

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The present study aimed to record all flower visitors of *Aechmea nudicaulis* (Bromeliaceae) and their foraging behaviour as well as their role in animal-plant and animal-animal relationships, and to assess temporal pattern of visitation on flowers. In order to estimate the influence of habitat and latitude on species richness and diversity, we compared the diversity and visitation frequency of flower visitors to *Ae. nudicaulis* in three different habitat types and of two sites about 2° of latitude apart (in Paraná and Santa Catarina). We recorded 40 taxa of flower visitors. A secondary forest in Paraná showed highest, restinga habitats on Santa Catarina Island lowest species richness and lowest visitation frequency. Main visitor groups were birds, bees and butterflies. The species spectrum differed considerably among habitats; species similarity was lowest between two secondary forest sites in Paraná and Santa Catarina. We recorded mainly hummingbirds (54.6% of all visits) as flower visitors in Paraná, whereas flowers in Santa Catarina were dominated by bees (44.5 – 59.7% of all visits). The temporal pattern of visits was similar at all sites, visits occurred mainly in the morning hours. However, there was no significant correlation between number of visits and hour of day. We showed that *Ae. nudicaulis* is visited by a highly diverse group of floral visitors. Habitat and latitudinal gradient had an influence on visitor species richness and spectrum. In contrast to the expectation that *Ae. nudicaulis* flowers may attract mainly hummingbirds (due to its floral morphology), we showed that the flowers are visited by a much wider spectrum of animal species, mainly insects and arachnids. Our findings answer the question what attracts animals to the inflorescences and allows to classify them.

Keywords: Brazil; animal-animal-interactions; flower visitors; habitat influence; species list; diel pattern; diversity indices

Introduction

Bromeliads (Bromeliaceae), a largely subtropical plant family with about 3000 species, are considered microhabitats for a wide range of animal species (Frank & Lounibos 2008). There are several studies assessing the association between particular groups of animals (e.g. aquatic invertebrates, especially mosquitoes), or the total assemblages of animals, and these plants (Picado 1913; Richardson 1999; Wittman 2000; Mestre et al. 2001; Frank et al. 2004; Müller & Marcondes 2007; Frank & Lounibos 2008).

Plant communities change among different habitats, also influencing the animal diversity and distribution (Bazzaz 1975). Due to differences in environmental factors such as vegetation density, vegetation age and complexity we supposed species spectra to differ between habitat types. Furthermore, the latitudinal gradient affects the diversity of animals and plants (Pianka 1966). So far, no studies have evaluated the influence of habitat or latitudinal differences on the spectrum of visitors to bromeliad flowers.

For the bromeliad *Aechmea nudicaulis* (L.) Grisebach (Bromeliaceae), the flowering phenology and the reproductive biology was recently studied in detail by Schmid et al. (2011). The pollinator spectrum was investigated in several studies, reporting in total 14 species of hummingbirds as visitors in the Atlantic rainforest in Paraná and São Paulo state (Araújo 1996; Buzato et al. 2000; Machado & Semir 2006; Piacentini & Varassin 2007; Cestari, 2009). Hummingbirds have been presumed to be the sole pollinators (Buzato et al. 2000; Machado & Semir 2006). However, for the euglossine bee *Euglossa annectans*, pollen of *Ae. nudicaulis* was found as larval food in cells (Cortopassi-Laurino et al. 2009) and Schmid et al. (2011) showed that besides hummingbirds certain species of bees can induce seed set in *Ae. nudicaulis* flowers. Hence it is clear that the plants are visited and can profit from visitors that do not fit into the often hastily classified floral syndromes, in this case ornithophily. Other studies on bromeliad flower visitors list only few visitor taxa; for some it is not clear whether this is because the visitor spectrum is indeed small (Sazima & Sazima 1999; Canela & Sazima 2003, 2005;), some chose only to report only parts of the visitor assemblage, *e.g.* hummingbirds (Krömer et al. 2006), and as of today, no study recorded all animal visitors associated with *Ae. nudicaulis* to make clear what the animals profit from visiting its flowers.

The temporal pattern that floral visitors follow in approaching flowers depends on factors like resource offering and interspecific/intraspecific competition (Morse 1980), and has an influence on pollen flow of cross-pollinated plants and therefore also on pollination efficiency of the visitor. The visitation frequency along the day seems to be related to reward availability (influenced by production rate as well as interspecific competition). The influence of resource offering on temporal pattern of visitors is little investigated for bromeliads (Canela & Sazima 2005).

From the bromeliad's view-point, pollinators and seed dispersers are the most important inflorescence visitors (Frank & Lounibos 2008), but not every flower visitor interacts in a mutualistic way. Some profit from the partnership without returning any benefit to the plant: robbers exploit floral resources by destroying floral tissue (e.g. gnawing holes) while thieves use the floral entrance and only bypass the reproductive organs (Inouye 1980). Herbivores (Strauss 1996) and visitors which predate on others, *e.g.* the pollinators, may also be seen as non-beneficial to the plants' reproduction. Nevertheless, all visitors are part of an interaction web surrounding the flowering plant and should profit from this association, but studies on flower visitors have mostly focused on only a part of the visitor assemblage, for example only on possible pollinators (Canela & Sazima 2003, 2005; Kaehler et al. 2005; Cestari 2009), investigating the issue which animal is important for the plant. With the present study, we tried to elucidate the importance of bromeliad flowers for the different guilds of floral visitors, and in which way these profit from flower visitation.

As part of a project studying the species richness of bromeliad flower visitors and the diversity and nature of their animal-plant interactions, this study aimed to (1) systematically record all flower visitors as well as their role in animal-plant and animal-animal relationships. Because habitat has proven to have an influence on species richness and diversity (Tews 2004), we (2) compared the diversity and visitation frequency of flower visitors to *Ae. nudicaulis* in three different habitat types, secondary forest, low- and high-vegetation restinga (sand dune habitat). Additionally, we tested the influence of the degree of latitude on species richness by comparing two sites about 2° of latitude apart. Finally we aimed to (3) assess diel pattern and foraging behaviour of different visitor groups.

Materials and methods

Study sites and study species

Field work was carried out in four Atlantic rainforest areas in coastal Santa Catarina (SC) and Paraná (PR), federal states in southern Brazil. On Santa Catarina Island, *Ae. nudicaulis* was studied in two different habitat types, secondary forest and dune vegetation (restinga, Sampaio et al. 2002). The secondary forest area (hereafter SC-F) was located at Santo Antônio de Lisboa (Zillikens et al. 2001; 27°30’26” S 48°30’28” W). Observations in restinga vegetation (hereafter SC-R) were performed at two sites. One of them, comprising Joaquina Beach (27°37’37” S, 48°26’59” W) and Campeche Beach (27°40’38” S, 48°28’48” W), is mainly covered with herbaceous plants and low trees up to 3 m (SC-R1). Another restinga site, in the RPPN Morro das Aranhas (Private Reserve of Natural Heritage, 27°28’11’’ S, 48°23’04’’ W, SC-R2) has an older restinga vegetation with higher trees (up to 10 m) than the first site and abundant/dense understorey vegetation. The fourth study area was located in the Rio Cachoeira Nature Reserve in Paraná State (25°18´51´´ S, 48°41´45´´ W), an Atlantic Rainforest Preservation Area, where afforested pasture areas are now covered with different stages of secondary forest (hereafter PR). Vegetation consisted of high trees (up to 20 m) and understorey plants.

Aechmea nudicaulis plants are found as facultative epiphytes growing on trees but also at ground level on rocks (Zillikens et al. 2001) or in the soil. In SC, we chose only terrestrial plants for our observations. In PR, where plants could only be found as epiphytes, we used only plants not growing higher than 5 m above ground. As flowers open only during daylight hours of a single day (Machado & Semir 2006, Schmid et al. 2011), one inflorescence observed on different days was counted as two independent inflorescence observation events because the flowers were different in position and number. Ants and flower mites visiting flowers and living within them have been previously studied (Schmid et al. 2010b) and were therefore mostly excluded from our observations.

Recording of flower visitors

To determine their spectrum, visitation frequency, diel pattern, and foraging behaviour, flower visitors of *Ae. nudicaulis* were observed directly or through binoculars. In order not to interfere with the visitation frequency, only few voucher specimens of insects were captured and deposited in the entomological collection of J. Steiner at the Native Bee Laboratory (LANUFSC), BEG, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil. Most of the visitors were identified *in situ* or later from photos or video captures taken in the field. Hummingbird species were determined using photographs and a species key (Grantsau 1988). Augochlorine bees were determined to genus level using the key in Silveira et al. (2002) and sorted into morphospecies. Observations in the field always started on the hour and lasted at least 60 min (range 1 – 8 h without break).

All animals touching a flower were counted as visitors. For every visitor, we recorded time of arrival, number of flowers visited, resource(s) collected or other aims for the visit, interactions with other visitors, and time of leaving the inflorescence. One inflorescence visit event was defined as to start with the first flower touched and to end with the visitor detaching from the last flower. For detailed information on sampling effort, observation time, and inflorescence numbers for the habitats see Table 1.

Relative species richness per taxon and habitat is expressed as the proportion (number of species in the habitat) / (number of all species recorded). Relative frequency of a visitor taxon is given as proportion of the taxon's visitation frequency in relation to the number of all visits recorded. Because the number of observed inflorescences and observation hours differed for each hour of day, the pattern of flower visitation during the anthesis of *Ae. nudicaulis* was determined by calculating the mean visitation frequency per hour and inflorescence for each hour of day on each date of observation. This resulted in 1 to 8 values per hour of day (depending on how often each hour was covered). The most abundant visitors, namely Trochilidae, Apoidea and Lepidoptera, were analysed separately; for SC, all other visitor taxa were grouped as "others", while for Paraná, vespid wasps were also frequent visitors and are therefore shown separately. The 'visitation diligence' of floral visitors during their visits was calculated as ratio of the number of flowers visited during one visit event by the number of open flowers at the visited inflorescence. Counted were the events of approaching a flower for each visitor regardless whether the flowers had already been probed during the same visit (thus allowing values >1).

Data analyses

For further analyses, the species list in Table 2 had to be modified so that it gave frequency values only on species level: unidentified specimens of a certain taxon were either distributed over all other species within that taxon (according to their observed frequency) or counted as an additional species. For the group Brachycera, neither of these approaches could be reasonably applied so this group was left out for the analyses (see Table S9).

To analyse species richness and diversity, we computed species accumulation curves (generated with the binomial mixture model by Colwell et al. (2004), using "EstimateS" and Suppl. Table 1), showing the number of species observed (Sobs), as well as the species richness estimator Chao2 and the number of uniques (species that have been recorded only once) over the cumulative number of observed flower visitors. The same modified table was used to calculate the Shannon and Simpson diversity indices using “EstimateS” (Colwell). Following the programme’s recommendation, for the site SC-R1 the classic formula for the Chao2 estimator was used instead of the bias-corrected version. For statistically comparing species richness among habitats, Sobs curves and the corresponding 95% confidence intervals were examined.

Species compositions were compared between the habitats by calculating three similarity indices: the Jaccard index, the complement of the turnover measure by Lennon et al. (2001), and the abundance-based estimator of the Jaccard index by Chao (Colwell EstimateS) (hereafter Chao-Jaccard). All three indices range from 0 (no similarity) to 1 (highest similarity). On two consecutive days, we recorded an exceptionally high number of individuals of *Bombus brasiliensis* (Apidae: Apinae: Bombini) at the same inflorescences in SC-F, probably because a nest of these social bees was near. To avoid errors produced by this artefact, for habitat comparisons, we therefore used two datasets, SC-F1 and -F2, where in SC-F2 these high *Bombus* frequencies were left out, although simply removing the visits of the *Bombus* workers from the dataset (as done for SC-F2) may not give the “normal” proportions of visits.

Values for the Jaccard and Lennon indices for F1 and F2 are equal because species abundances are not taken into account, in contrast to the Chao-Jaccard index. For similarity analysis we also used the modified species table in Table S9.

The Wilcoxon matched-pairs signed-ranks test, performed in JMP®, was used to test differences of visitation rates among the hummingbird species in PR as well as differences of total visitor numbers per hour among the different habitats. P-values of all tests were corrected using the sequential Bonferroni method (Holm 1979).

Table 1: Detailed information about sampling effort in the four habitats on Santa Catarina Island, SC and Paraná.

		n days	n inflorescences	total observation time [h]	observation period	observation start
SC-F	Nov 05 – Nov 07	14	30	55	05:00 – 17:00	05:00 – 14:00
SC-R1	Nov 05 – Nov 07	10	27	35	05:00 – 15:00	05:00 – 11:00
SC-R2	Nov 05 – Nov 07	6	21	25	07:00 – 16:00	07:00 – 11:00
PR	Oct – Nov 07, Dec 08	13	19	72	05:00 – 17:00	05:00 – 14:00

Results

Species richness and spectrum

Taking together all sites, we recorded at least 40 taxa, 5 bird and 35 arthropod taxa (insects and spiders) as flower visitors of *Ae. nudicaulis* (Table 2). In addition, we registered one hummingbird and three arthropod taxa outside regular observation sessions (see * in Table 2). In the following analyses we take only the regularly assessed visits into account, leaving the additionally records aside. Highest species richness, 20 species, was observed in the secondary forest habitats of Paraná and Santa Catarina, lowest species richness was observed in the two restinga habitats SC-R1 and SC-R2 (14 species each) (Figure 1A).

Statistical analyses of species richness (Figure 2A-E) showed that for the two habitats SC-R2 and SC-F1/F2 the observed species (Sobs) curves converged with the species number predicted by the species richness estimator Chao 2. Three out of five species accumulation curves (those for SC-F1, -F2, and SC-R2) reached a plateau, and that for SC- R1 would most probably have reached a plateau after little additional sampling because the number of uniques already began to decrease. Only for the secondary forest in PR none of the curves did reach a plateau yet: the species richness estimator predicted a much higher (and most likely underestimated because still increasing) species number than observed and the number of uniques was still increasing. The two Sobs curves for F1 and F2, only differing in the presence or absence of extreme high numbers of bumble bees observed on two consecutive days, reached the same species number. Only the sampling effort to reach the plateau differed between them. Both the accumulation curves and the Chao2 estimator indicate that species richness was highest in PR and lowest in the restinga habitats (Figure 2F-J). For each habitat, the curves of the Shannon and the Simpson index stabilized indicating that even with increased sampling effort there would have been no change anymore.

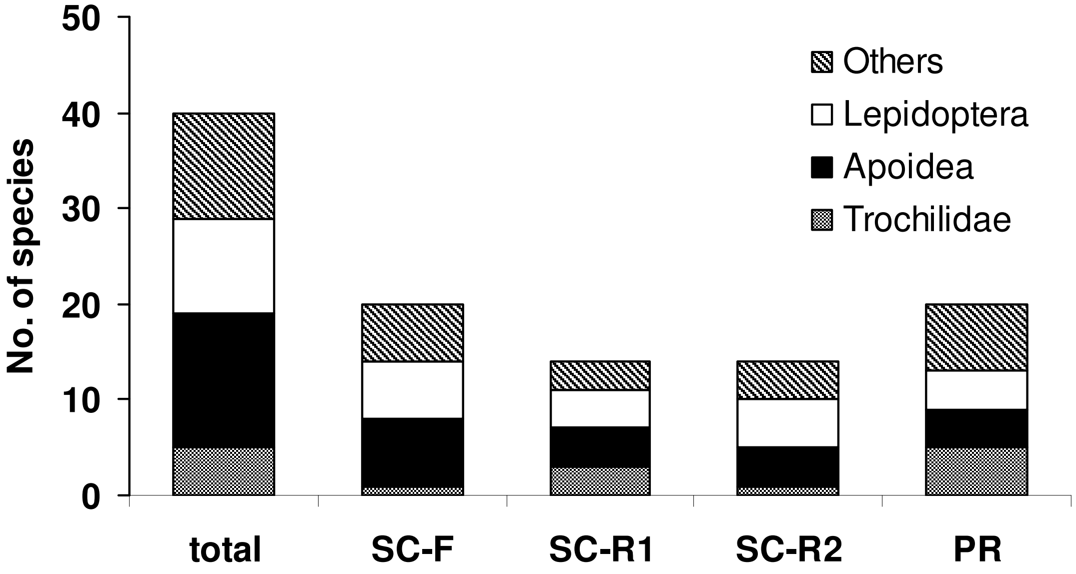
The number of species of floral visitors was significantly different between PR and the habitats on Santa Catarina Island, shown by the Sobs curves for SC-F1/F2 and SC-R1/R2 leaving the 95% confidence interval of the PR Sobs curve. There was also a significant difference between forest and restinga habitats in SC (Figure S1A-E).

Table 2: Species spectrum, visitation frequency and richness of flower visitors of Aechmea nudicaulis in different habitat types (secondary forest and restinga) and different geographic regions [situation] (Santa Catarina and Paraná). Visitation frequency (%) is given as percentage of total visitor number observed on flowers. Abbreviations: N = total number observed, n = nectar, p = pollen, h = herbivore, en = extrafloral nectar, r = predator, x = recorded in the habitat but no frequency available. * Individuals were observed as visitors but not during regular observation sessions so there is no frequency available. *Aphantochroa cirrochloris* was observed as visitor, but only in urban areas on SC Island.

Visitor taxon	**Visitation frequency**										**Behavior**
	All sites		SC Island						Paraná		
			forest		restinga 1		restinga 2		forest		
	%	N	%	N	%	N	%	N	%	N	
Trochilidae	**22.81**	**(450)**	**3.73**	**(39)**	**32.81**	**(42)**	**11.04**	**(17)**	**54.57**	**(352)**	
Amazilia fimbriata	1.37	(27)	-	-	12.5	(16)	-	-	1.71	(11)	n
Amazilia versicolor	4.66	(92)	-	-	-	-	-	-	14.26	(92)	n
Anthracothorax nigricollis	9.28	(183)	-	-	-	-	-	-	28.37	(183)	n
*Aphantochroa cirrochloris**		x	-	-	-	-	-	-	-	-	n
Thalurania glaucopis	7.40	(146)	3.73	(39)	20.31	(26)	11.04	(17)	9.92	(64)	n
Trochilidae n. ident.	0.10	(2)	-	-	-	-	-	-	0.31	(2)	n
Thraupidae	**0.51**	**(10)**	-	-	**6.25**	**(8)**	-	-	**0.31**	**(2)**	
Coereba flaveola	0.51	(10)	-	-	6.25	(8)	-	-	0.31	(2)	n
Apoidea	**63.20**	**(1247)**	**86.90**	**(909)**	**44.53**	**(57)**	**59.74**	**(92)**	**29.30**	**(189)**	
Apidae											
Apis mellifera	0.05	(1)	-	-	-	-	-	-	0.16	(1)	p
Bombus brasiliensis	29.90	(590)	56.41	(590)	-	-	-	-	-	-	n + p
Ceratina (Crewella) sp.1	0.35	(7)	0.67	(7)	-	-	-	-	-	-	p
Ceratina (Crewella) sp.2	4.46	(88)	-	-	-	-	-	-	13.64	(88)	p
Euglossa annectans	3.55	(70)	5.35	(56)	-	-	9.09	(14)	-	-	n + p
Meliponini	0.81	(16)	-	-	-	-	-	-	2.48	(16)	p
Plebeia droryana	1.72	(34)	3.06	(32)	1.56	(2)	-	-	-	-	p
*Tetragonisca angustula**	-	-	-	-	-	-	-	-	-	x	p
Trigona spinipes	1.06	(21)	-	-	16.41	(21)	-	-	-	-	p
Xylocopa brasilianorum	2.13	(42)	1.05	(11)	5.47	(7)	15.58	(24)	-	-	n
Xylocopa artifex	4.51	(89)	8.32	(87)	-	-	1.30	(2)	-	-	n
Xylocopinae n.ident.	2.08	(41)	1.91	(20)	14.84	(19)	1.30	(2)	-	-	n
Apidae n. ident.	0.25	(5)	-	-	-	-	0.65	(1)	0.62	(4)	

Visitor taxon	Visitation frequency										Behavior
	All sites		SC Island						Paraná		
			forest		restinga 1		restinga 2		forest		
	%	N	%	N	%	N	%	N	%	N	
Halictidae											
Augochlora (*A.*) sp. 1	-	-	-	-	-	x	-	-	-	-	p
Augochlora (*A.*) sp. 2	4.05	(80)	-	-	-	-	-	-	12.40	(80)	p
Augochlora (*A.*) sp. 3	5.37	(106)	10.13	(106)	-	-	-	-	-	-	p
Augochlorella ephyra	-	-	-	-	-	-	-	x	-	-	p
Augochlorini n. ident.	2.89	(57)	-	-	6.25	(8)	31.82	(49)	-	-	p
Lepidoptera	**7.40**	**(146)**	**7.65**	**(80)**	**10.16**	**(13)**	**18.18**	**(28)**	**3.88**	**(25)**	
Castniidae											
Imara pallasia	1.42	(28)	2.58	(27)	0.78	(1)	-	-	-	-	n
Synpalamides phalaris	0.76	(15)	0.57	(6)	-	-	5.84	(9)	-	-	n
Geyeria decussata	0.51	(10)	0.29	(3)	-	-	4.55	(7)	-	-	n + p
Pieridae											n
Pieridae (*Phoebis* sp.)	0.41	(8)	0.29	(3)	1.56	(2)	-	-	0.47	(3)	n
Lycaenidae											
Strymon ziba	0.86	(17)	0.29	(3)	7.03	(9)	3.25	(5)	-	-	n + o
Strymon sp.	0.05	(1)	-	-	-	-	-	-	0.16	(1)	n
Heliconiidae											
Heliconius ethilla	2.84	(56)	3.44	(36)	0.78	(1)	-	-	2.95	(19)	n + p
*Heliconius erato**	-	-	-	x	-	-	-	-	-	-	n
Hesperiidae											
Hesperiidae sp.1	0.15	(3)	-	-	-	-	1.95	(3)	-	-	n
Lepidoptera sp. 1	0.05	(1)	-	-	-	-	-	-	0.16	(1)	n
Lepidoptera sp. 2	0.20	(4)	-	-	-	-	2.60	(4)	-	-	n
Lepidoptera n. ident.	0.15	(3)	0.19	(2)	-	-	-	-	0.16	(1)	n
Heteroptera	**0.30**	**(6)**	**0.57**	**(6)**	-	-	-	-	-	-	
Miridae sp. 1	0.10	(2)	0.19	(2)	-	-	-	-	-	-	h
Reduviidae sp. 1	0.20	(4)	0.38	(4)	-	-	-	-	-	-	pr
Coleoptera	**0.96**	**(19)**	**0.48**	**(5)**	-	-	**5.19**	**(8)**	**0.93**	**(6)**	
Curculionidae sp. 1	0.30	(6)	0.48	(5)	-	-	-	-	0.16	(1)	h
Curculionidae sp. 2	0.46	(9)	-	-		-	5.19	(8)	0.16	(1)	h
Curculionidae sp. 3	0.10	(2)	-	-		-	-	-	0.32	(2)	h
Chrysomelidae sp. 1	0.05	(1)	-	-	-	-	-	-	0.16	(1)	h
Coleoptera n. ident.	0.15	(1)	-	-	-	-	-	-	0.16	(1)	
Diptera	**1.27**	**(25)**	**0.29**	**(3)**	**3.91**	**(5)**	**3.90**	**(6)**	**1.71**	**(11)**	
Brachycera n. ident.	1.27	(25)	0.29	(3)	3.91	(5)	3.90	(6)	1.71	(11)	en
Orthoptera	**0.05**	**(1)**	-	-	-	-	**0.65**	**(1)**	-	-	
Caelifera	0.05	(1)	-	-	-	-	0.65	(1)	-	-	h

Visitor taxon	**Visitation frequency**										**Behavior**
	All sites		SC Island						Paraná		
			forest		restinga 1		restinga 2		forest		
	%	N	%	N	%	N	%	N	%	N	
Vespidae	**3.19**	**(63)**	**0.29**	**(3)**	**0.78**	**(1)**	**-**	**-**	**9.15**	**(59)**	**p + n**
Vespidae sp. 1	0.20	(4)	0.29	(3)	0.78	(1)	-	-	-	-	
Vespidae sp. 2	2.99	(59)	-	-	-	-	-	-	9.15	(59)	
Blattaria	**-**	**-**	**-**	**-**	**-**	**x**	**-**	**-**	**-**	**-**	**h**
Blattaria*	-	-	-	-	-	x	-	-	-	-	h
Araneae	**0.30**	**(6)**	**0.10**	**(1)**	**1.56**	**(2)**	**1.30**	**(2)**	**0.16**	**(1)**	
Salticidae	0.30	(6)	0.10	(1)	1.56	(2)	1.30	(2)	0.16	(1)	pr
Total species number		**40**		**20**		**14**		**14**		**20**	



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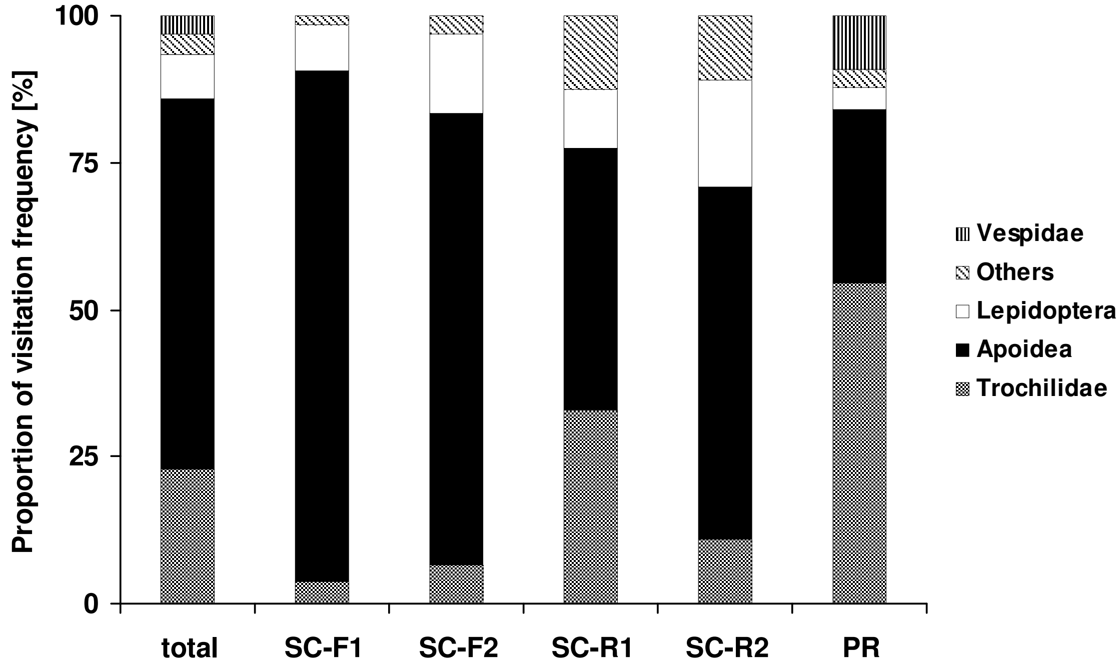
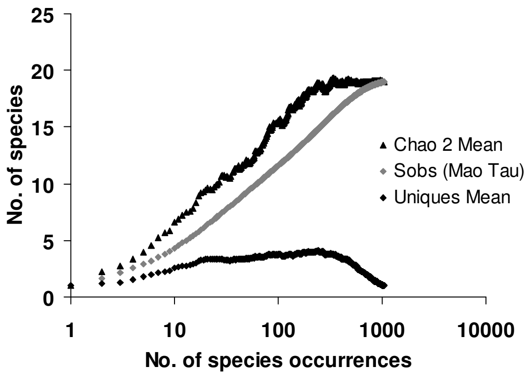


B

Figure 1. Species richness (**A**), percentage of species richness (**B**) and visitation frequency (**C**) of flower visitors on *Aechmea nudicaulis* was different for the three sites PR, SC-R, and SC-F, Paraná and Santa Catarina Island, southern Brazil.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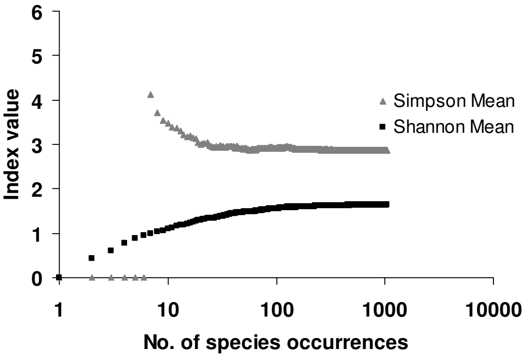
No. of species occurrences
▲ Chao 2 Mean
◆ Sobs (Mao Tau)
◆ Uniques Mean

A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■ Shannon Mean

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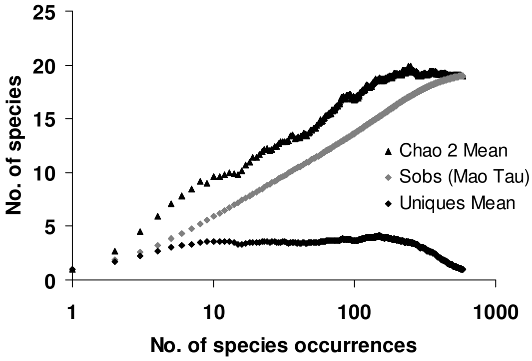
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No. of species occurrences
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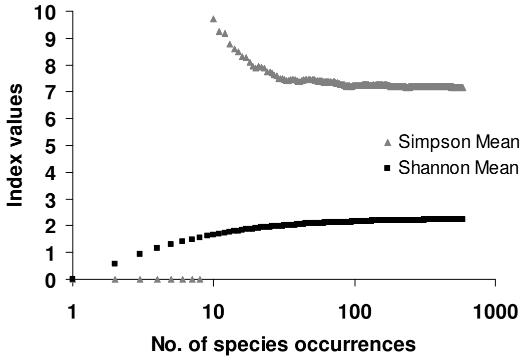
◆ Sobs (Mao Tau)
◆ Uniques Mean

B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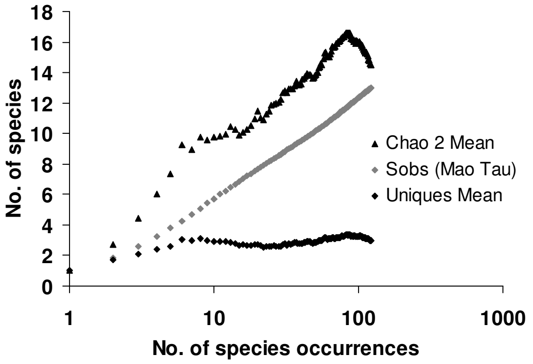
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No. of species occurrences
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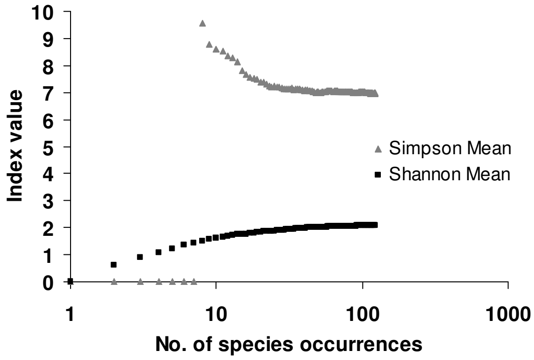
◆ Sobs (Mao Tau)
◆ Uniques Mean

C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No. of species occurrences
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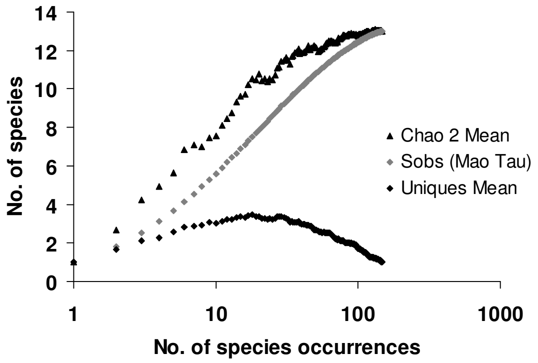
■ Shannon Mean

H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No. of species occurrences
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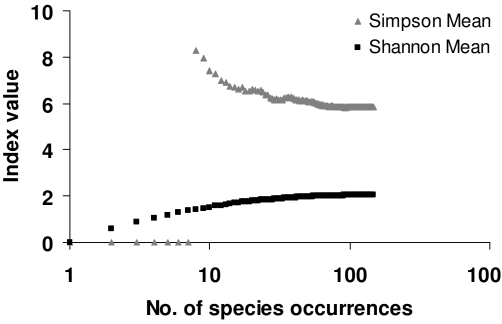
◆ Sobs (Mao Tau)
◆ Uniques Mean

D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No. of species occurrences
▲ Simpson Mean
■ Shannon Mean

I

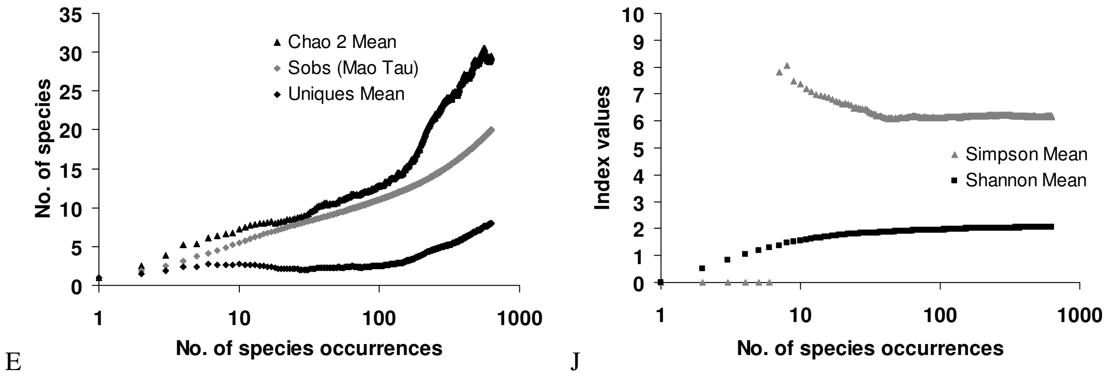


Figure 2. Species accumulation curves (A-E) and diversity indices Shannon and Simpson (F-J) (Sobs = number of species observed) for *Aechmea nudicaulis*, in SC-F1 (A/F), SC-F2 (B/G), SC-R1 (C/H), SC-R2 (D/I), and PR (E/J), and development of the nonparametric species richness estimator prediction Chao2. Uniques are shown additionally. X-axis is scaled logarithmically. SC-F1 = secondary forest on Santa Catarina Island; SC-F2 = data set as SC-F1 but without *Bombus brasiliensis*; SC-R1 = low vegetation restinga on Santa Catarina Island; SC-R2 = high vegetation restinga on Santa Catarina Island; PR = secondary forest in the litoral of Paraná, southern Brazil.

The three main visitor groups were bees, hummingbirds and butterflies. These groups were equally diverse in PR (four species each), bees were most diverse in SC-F (seven species), and bees and lepidopterans contained the highest number of species (four species each) in both SC-R1 and R2 (Figure 1A). The spectrum of species differed considerably among habitats (Table 2). All three similarity indices, Jaccard, Lennon and Chao-Jaccard, indicated that similarity was lowest between PR and SC-F1 and between SC-R2 and SC-F2 and highest between SC-R1 and SC-F1 (Figure 3).

On Santa Catarina Island, we observed two species of hummingbirds, *Thalurania glaucopis* and *Amazilia fimbriata,* attending inflorescences of *Ae. nudicaulis* (Table 2). Bees attending flowers belonged to four tribes in two families (Apidae: Apini, Xylocopini, Ceratinini; Halicitidae: Augochlorini) and most of the recorded bees were females. Common genera at both habitats in SC were *Xylocopa*, *Ceratina* and *Augochlora.* In the secondary forest of Paraná, the spectrum of hummingbird species differed, comprising *Anthracothorax nigricollis* and *Am. versicolor* in addition to the other two species recorded in SC. *Coereba flaveola* (Thraupidae) attended flowers in both SC-R1 and PR. All bee species recorded in PR were small bees like *Augochlorini* and *Ceratina.* In contrast, on Santa Catarina Island we observed small (*Augochlora, Plebeia*) and large bee species like *E. annectans*, *B. brasiliensis,* and *X.* spp. Butterflies of the families Nymphalidae, Pieridae, Lycaenidae, and Hesperiidae were recorded as flower visitors. At the sites in SC, butterflies and moths were even as diverse as bees (Figure 1A). Beetles, grasshoppers, and cockroaches were recorded with only few species. Other visitor groups of *Ae. nudicaulis* flowers were reduviid and mirid bugs, flies, wasps, and jumping spiders. *Thalurania glaucopis* and *H. ethilla* were the only visitor species recorded in all three habitats (Table 2).

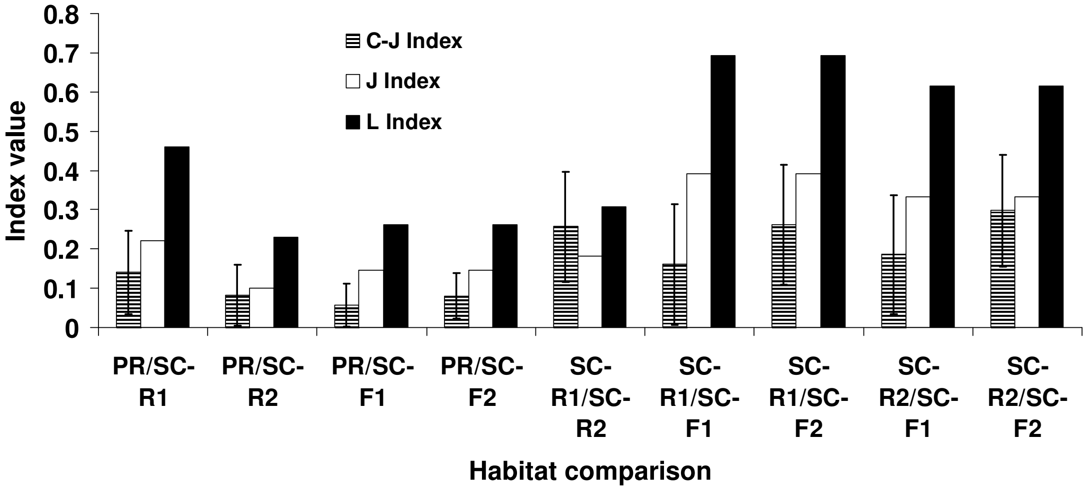


Figure 3. Similarity indices for pair-wise habitat comparisons. The classic Jaccard and the Lennon index show equal values for the comparisons of SC-F1 and SC-F2 with the other habitats because both F1 and F2 differ only in one visitor frequency but not in species number. Only the Chao-Jaccard index differs between the two SC forest habitats because it takes frequencies into account.

Visitation frequency of flower visitors

Mean visitation frequency per hour and inflorescence differed between sites. In PR and SC-F it was significantly higher than in SC-R throughout the day (Figure 4). In Paraná, hummingbirds were the most frequent visitor group with 54.6% of all observed visits (Figure 1B, Table 2). Among them, *An. nigricollis* stood out in overall visitation frequency (52.3% of all hummingbird visits, Table 2). *Amazilia fimbriata* was the significantly least frequent hummingbird and did not regularly visit the flowers (Figure 5A; Table 3). The second most frequent visitor group were bees, followed by wasps (Table 1, Figure 1B). At the SC sites bees were the most frequent visitors in both secondary forest and restinga sites (SC-F 86.9% / SC-R1 44.5% / SC-R2 59.7% of all visits recorded, Figure 1B). The extremely high number of visits of *B. brasiliensis* (457 specimens / 4 observation hours) observed on two consecutive days at the same inflorescences in secondary forest on Santa Catarina Island (SC-F1) resulted in a proportion of bee visits of 86.9%, but even omitting the data of those two days (SC-F2) the frequency was still high (76.8% of all visits). Butterflies and moths were the third frequent flower visitor group (Figure 1B). Among them, *H. ethilla* was most frequent, followed by *Imara pallasia* and *Strymon ziba*. Other taxa (Vespidae, Diptera, and Coleoptera) were moderately frequent.

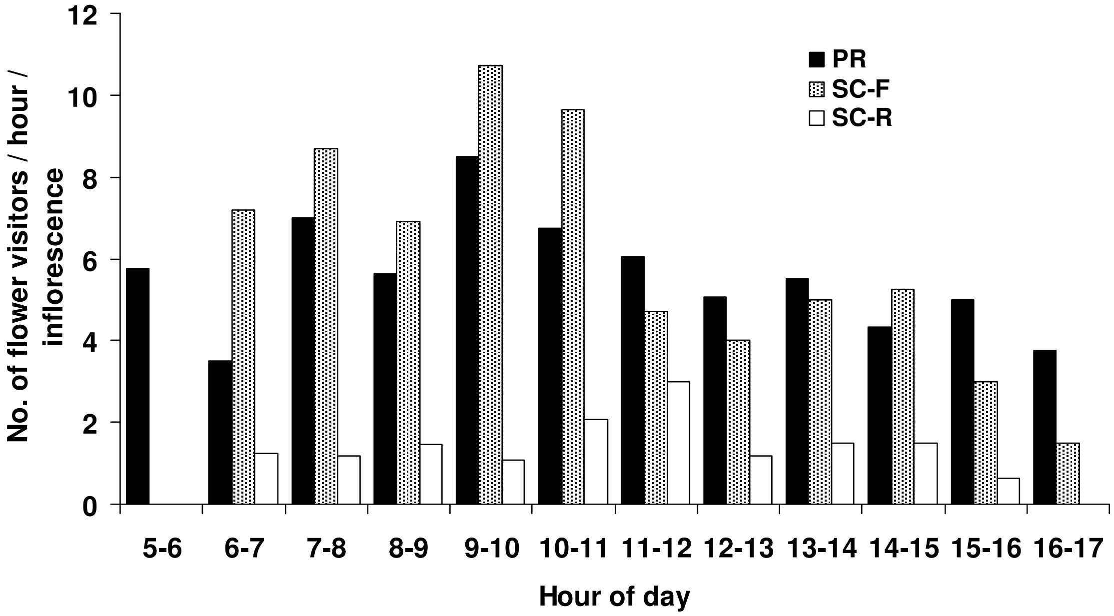
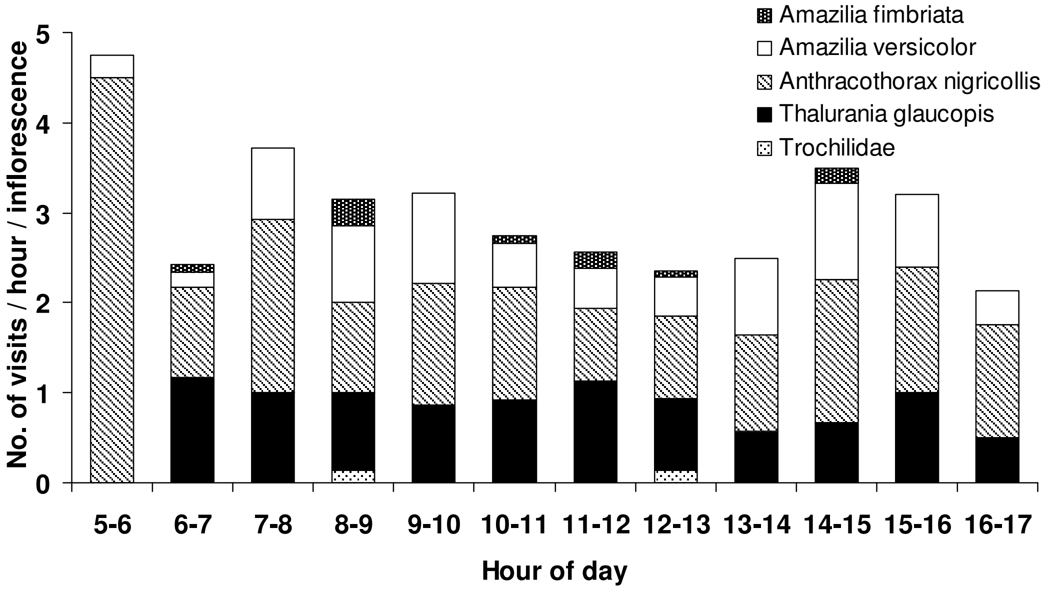


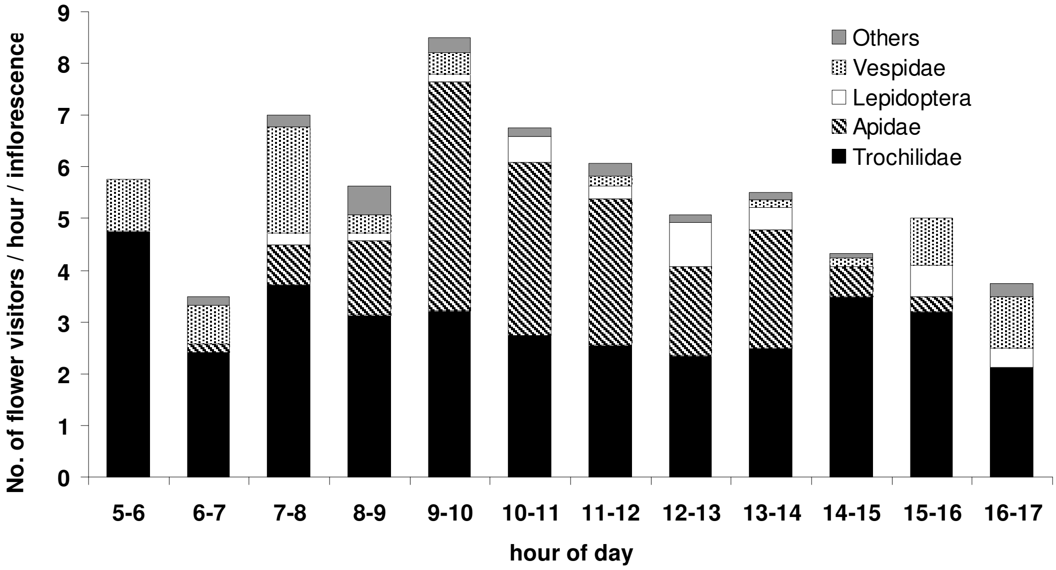
Figure 4. Diel pattern flower visitors on *Aechmea nudicaulis* for forest and restinga sites on Santa Catarina Island and forest site in Paraná, southern Brazil.

Diel pattern of floral visitors

Tendencies of diel pattern were similar at all sites with frequencies peaking between 9:00 and 12:00 h. In forests in PR and SC, the overall per-hour visitation frequency rose until 10:00 h. In the restinga habitats on Santa Catarina Island (SC-R) it was much lower than in secondary forest in both SC and PR (Figure 4) and reached a peak at 12:00 h. We found no significant correlation between hour of day and number of flower visitors in all habitats, as well as for hour of day and number of hummingbird visits in PR (Table 3).



A



B

Figure 5. Diel pattern of of hummingbirds (**A**) and all flower visitors (**B**) in Paraná, southern Brazil.

In PR, visitation frequency of bees peaked between 9:00 and 10:00 h (Figure 5B). Bees foraged on the flowers between 06:00 and 16:00 h. From 09:00 to 12:00 h bees were even more frequent than hummingbirds (Figure 5B). The hummingbirds *An. nigricollis, T. glaucopis*, and *Am. versicolor* have similar daily foraging patterns without a clear peak except for *An. nigricollis* which was most frequent in the first daylight hour. It was also significantly more frequent than the other three species (Table 3, Figure 5A).

Table 3: Statistical tests used to compare visitation frequencies among habitats and among hummingbirds in PR (Wilcoxon matched-pairs signed-ranks test) as well as to correlate visitation rates with hour of day (Spearman-correlated (ρ)).* P values significant ($\alpha = 0.05$) after sequential Bonferroni correction. Af = *Amazilia fimbriata*, Av = *Amazilia versicolor*, An = *Anthracothorax nigricollis*, Tg = *Thalurania glaucopis*. F1/F2 = SC-F1/SC-F2, R1/R2 = SC-R1/R2.

	Wilcoxon Matched-Pairs Signed-Ranks Test	
Comparison	Signed-rank test statistic	P
PR-R1	27.5000	0.0020*
PR-R2	22.5000	0.0039*
PR-F1	-2.0000	0.9097
PR-F2	22.0000	0.0923
R1-R2	-9.0000	0.2500
R1-F1	22.5000	0.0039*
R1-F2	-22.5000	0.0039*
R2-F1	-22.5000	0.0039*
R2-F2	-22.5000	0.0039*
An-Af	39.0000	0.0005*
An-Av	39.0000	0.0005*
An-Tg	32.0000	0.0083*
Af-Av	-39.0000	0.0005*
Af-Tg	-33.0000	0.0010*
Av-Tg	-11.5000	0.3311

	Spearman's rho	P
hour-total visitors PR	-0.4266	0.1667
hour-total visitors F1	-0.3636	0.2453
hour-total visitors F2	-0.2028	0.5273
hour-total visitors R1	0.3891	0.2665
hour-total visitors R2	-0.4519	0.2220
hour-hummingbird PR	-0.4196	0.1745

In SC-F, bees dominated on the flowers almost all day long; only in the afternoon butterflies and moths overruled them (Figure 6A). Most visitations occurred in the morning hours till 11:00 (Figure 6). In the morning, bees were also the most frequent visitors in both SC-R1 and -R2; around midday there were more hummingbirds, and later in the afternoon, butterflies and moths were most frequent (Figure 7A-C).

In PR, there were more visitors collecting nectar than pollen at all times of the day, but a peak of pollen collectors occurred around midday (Figure 8A). In SC, frequency of pollen collectors was highest in the first hours of anthesis (Figure 8B), even exceeding that of nectar gathering visitors, and strongly declining after 8:00 h. Bees alone showed the same pattern: they collected both nectar and pollen, the latter mainly in the morning, the former after the pollen had been depleted (Figure 8C).

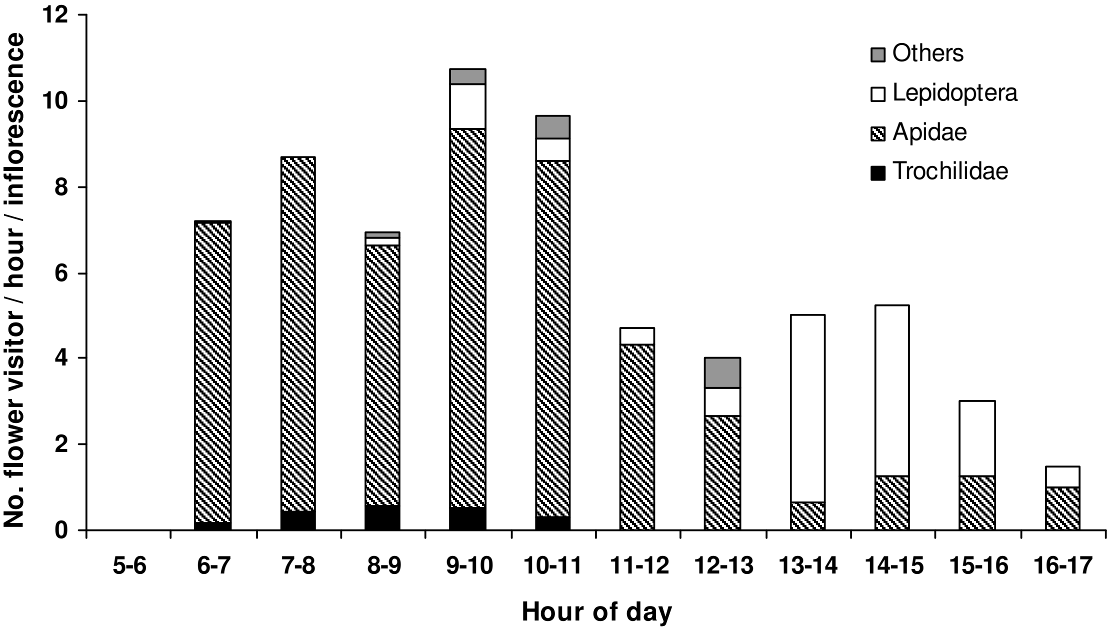
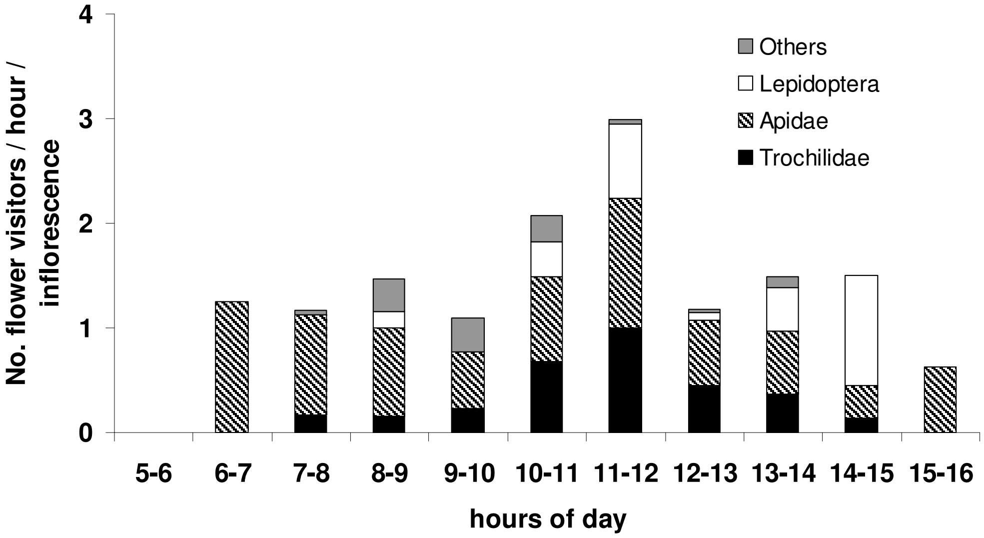


Figure 6. Diel pattern of all flower visitors on *Aechmea nudicaulis* flowers (**A**; SC-F1) and without the two extreme *Bombus*-rich observation units (**B**; SC-F2) in secondary forest on Santa Catarina Island.



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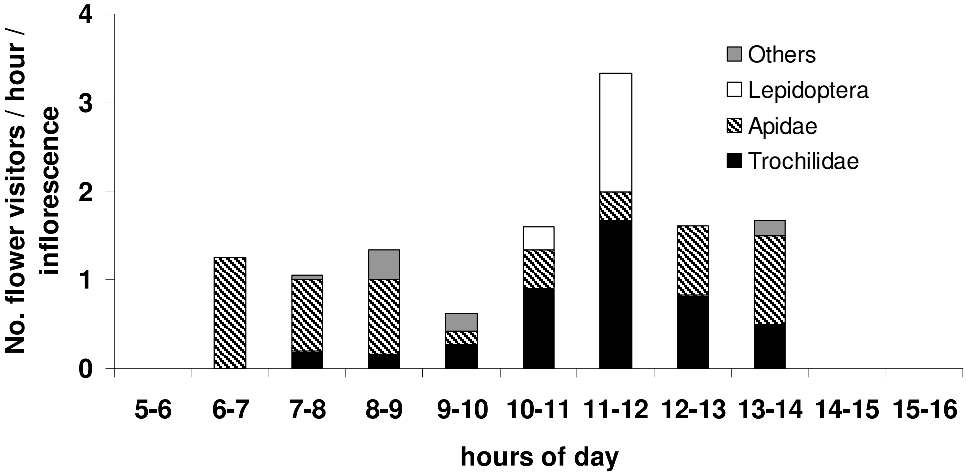

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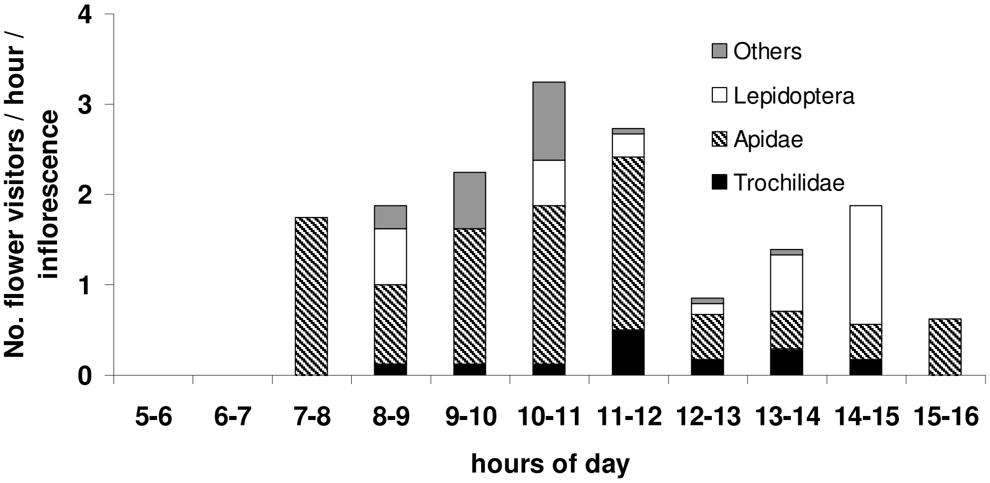

C

Figure 7. Diel pattern of flower visitors on *Aechmea nudicaulis* flowers in restinga areas of Santa Catarina Island, (**A**) R1 and R2 together, (**B**) R1 and (**C**) R2.

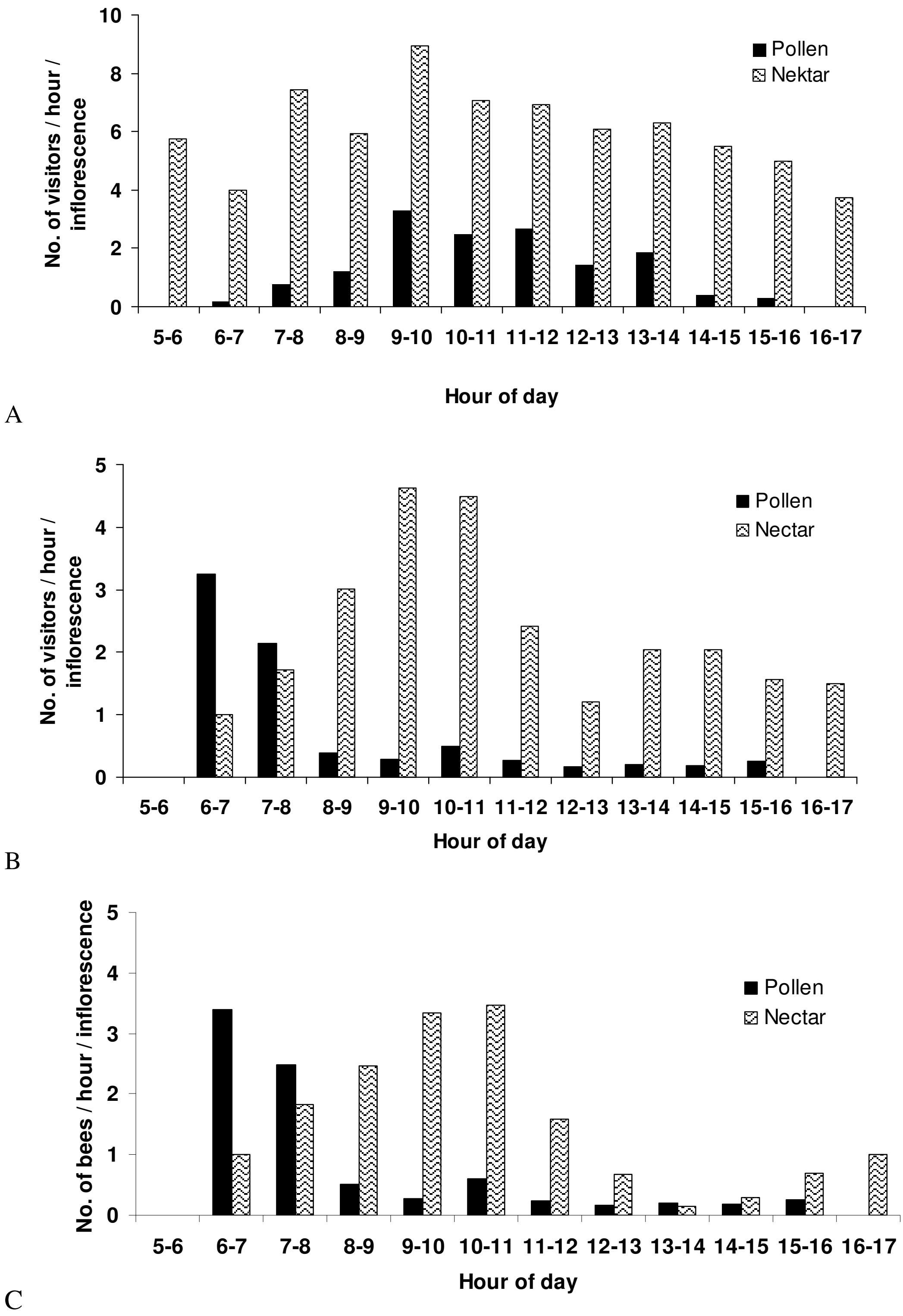

Figure 8. Foraging aim of flower visitors on *Aechmea nudicaulis* flowers in Paraná (**A**), on Santa Catarina Island (**B**), and for bees in SC (**C**), southern Brazil.

Foraging behaviour

During almost every visit, hummingbirds inserted their bills into all open flowers available on an inflorescence while hovering in front of the flowers (Figure 9A-C, Video S2/S3). In contrast, *Co. flaveola* drank floral nectar while sitting on the inflorescence (Figure 9D).

Behaviour on the flowers differed considerably between bee species. *Euglossa annectans* females inserted their long proboscides into the flowers taking up nectar and/or pollen (Figure 9E; Video S4); their heads stayed outside, thus remaining free from pollen. When they collected pollen, they left the flowers several times for a short period to groom the pollen grains off their proboscides while hovering next to the flowers (Video S4). Only few *Euglossa* males were observed visiting flowers; they always collected nectar (Video S5). Carpenter bee (Apidae: Xylocopinae) females of the species *X. artifex* (Fig 9F, Video S6) and the larger *X. brasilianorum* mainly collected nectar, pushing their heads into the flowers to reach the nectar chamber at the corolla base. Small carpenter bees of the genus *Ceratina* collected only pollen because they did not reach the nectar chambers. Pollen was collected with the forelegs and then transferred to scopae on the hind legs. *Bombus brasiliensis* workers collected both nectar and pollen. They entered the flowers with half of their heads to reach the nectar chamber (Figure 9G, Video S7). Pollen was collected with the forelegs and groomed during flight. *Plebeia droryana* workers entered the flowers with half of their bodies, collected pollen with their forelegs and stored it in their corbiculae but they did not contact the stigma. Nectar was out of reach for them. *Trigona spinipes* (Figure 9H) as well as *P. droryana* workers also occasionally licked extrafloral nectar at the sepal spines on the outer face of the flowers. Augochlorine sweat bees collected mainly pollen with their forelegs and stored it on their hind legs and the abdomen (Figure 9I). To reach the nectar with extended proboscides, the slender bees entered the flowers with half of their bodies (Video S8).

The two heliconiine species *H. ethilla* (Figure 9J) and *H. erato* (Figure 9K) collected nectar and pollen. The latter was accumulated as yellow mass at the bases of their proboscides (Figure 9J, white arrow). Castniid moths collected nectar from flowers while perching on the inflorescences for several minutes or even some hours (Figure 9L-O) also accumulating pollen at the bases of their proboscides (Figure 9N). Lycaenid (Figure 9P) and hesperiid (Figure 9Q) butterflies visited flowers to collect extrafloral (Figure 9P, arrow) and floral nectar, entering the flowers only with their proboscides while sitting on the inflorescences.


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Figure 9. Flower visitors of *Aechmea nudicaulis* in SC and PR: *Anthracothorax nigricollis* (**A**) and *Amazilia versicolor* (**B**) visiting flowers in Cachoeira Reserve, Paraná, Brazil, December 2008. *Thalurania glaucopis* female (**C**), UCAD, SC-F. *Coereba flaveola* (**D**) sitting on an inflorescence in Campeche Beach. *Euglossa annectans* (**E**) drinking nectar only entering the flower with its proboscis. *Xylocopa artifex* (**F**) drinking nectar. *Bombus brasiliensis* (**G**) collecting nectar and pollen. *Trigona spinipes* (**H**) licking up extrafloral nectar. *Augochlora* sp.2 (**I**) with pollen on hind legs and abdomen. *Heliconius ethilla* (**J**) and *Heliconius erato* (**K**) drinking nectar and with pollen gathered at the proboscis (white arrow in J). *Imara pallasia* (**L**) on an inflorescence, showing the colourful wing pattern. *Geyeria decussata* (**M**) drinking nectar, in (**N**) with pollen gathered at the proboscis (white arrow). *Synpalamides phalaris* (**O**) drinking nectar *Strymon ziba* (**P**) drinking from a droplet of extrafloral nectar (white arrow). Hesperiid butterfly (**Q**) in SC-R2 on flowers. Leafcutter ants (*Acromyrmex* sp.) (**R**) cutting petals, anthers, and stigmas of flowers along their foraging route. Curculionid beetle (**S**), another beetle (**T**), feeding on withered flowers, and a cockroach (**U**) on a bud. Mirid bug (**V**) piercing a flower with its rostrum (arrow).

Although ants were not recorded systematically, we observed one remarkable event when leafcutter ants of the genus *Acromyrmex* (Figure 9R) were cutting petals, anthers and stigmas from open flowers of one inflorescence being part of their foraging route. Beetles and cockroaches were recorded as herbivores on the inflorescences causing light to severe damage by feeding on flowers or unripe fruits (Figure 9S-U). Grasshoppers fed on open flowers or withered petals. Vespid wasps and flies were observed walking on the inflorescences licking up extrafloral nectar from the sepals. A mirid bug was observed piercing floral tissue with its rostrum, apparently sucking plant sap or floral nectar (Figure 9V).

Flower visitation rate (‘visitation diligence’) differed between visitor species as well as between visitations within a species. Hummingbirds had the highest ratios (mean: 0.85, range 0.08 - 2,86), followed by bees (Figure 10). Least diligent were the heliconiine butterflies with a ratio of 0.28 (range 0.05 – 0.80).

Animal-animal interactions

On inflorescences of *Ae. nudicaulis*, we observed individuals of different species foraging simultaneously on flowers without aggression, but agonistic behaviour could also be observed. In Cachoeira Natural Reserve (PR), individuals of the hummingbird species *An. nigricollis* showed distinct territorial behaviour. A bird was sitting near the tree with the flowering plants almost all day long. It attacked approaching hummingbirds (regardless of species) and gave rapid series of high and monotonous buzzy notes (Stiles 1982) while chasing the intruders over a short distance. After that the bird came back to resume its sitting place.

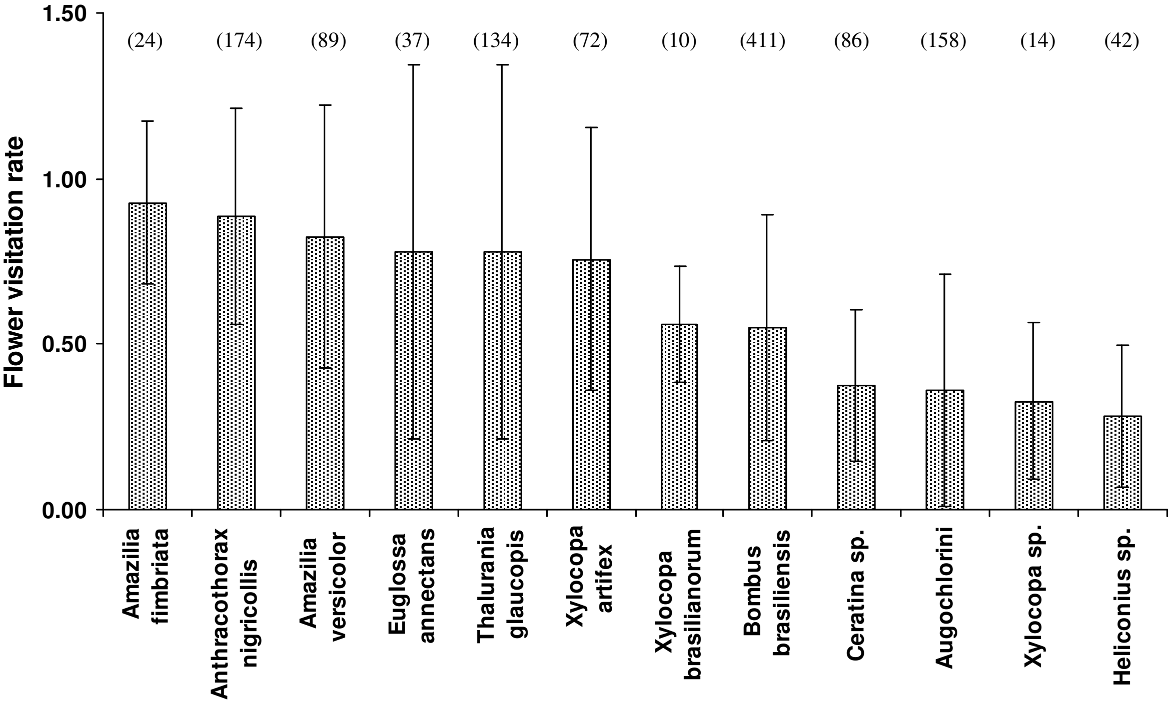

Figure 10. Mean flower visitation rate (number of visited flowers divided by number of available flowers) of visitors on *Aechmea nudicaulis* flowers. Number of flowers visitation events given in brackets. Values >1 indicate that at least one flower was touched twice during one visitation event.

In secondary forest on Santa Catarina Island, we observed *Bombus* und augochlorine bees together on the same inflorescences, but the latter kept some distance to the former. Approaching *Xylocopa* bees drove ants away (perhaps *Crematogaster* sp.) that foraged for extrafloral nectar, whereas other, larger ants (perhaps *Camponotus* sp.) attacked and dispelled smaller bees (*Augochlora*, *Plebeia*). *Euglossa* bees behaved very timidly while approaching and foraging on *Aechmea* flowers and were easily chased away by larger bees, butterflies, moths, and birds.

Several individuals of the moth species *Imara pallasia* (Castniidae) showed some kind of aggressive behaviour: one specimen remained on an inflorescence for several hours and collected nectar. When another moth or a bee tried to approach the inflorescence, the sitting *Imara* repeatedly opened the wings, exposing the brightly coloured hindwing pattern (Figure 9L). In contrast, two *Geyeria decussata* (Castniidae) individuals were observed foraging together on the same inflorescence together without any signs of aggression.

A reduviid bug lurking on the inflorescence attacked a euglossine bee several times but did not succeed in capturing the bee.

Discussion

Flower visitor species richness

This study is the first approach to record all animals associated with *Ae. nudicaulis* inflorescences in a range of habitats and sites. It revealed the high number of at least 40 associated species. Some floral visitors were already known from earlier studies. In Buzato et al. (2000), besides *Th. glaucopis*, six other hummingbird species not observed by us have been reported as visitors of *Ae. nudicaulis* flowers in the Atlantic forest in São Paulo state. Three hummingbird species are known to visit *Ae. nudicaulis* flowers in Paraná (Araújo 1996; Piacentini & Varassin 2007) and another three species were visitors of *Ae. nudicaulis* in Trinidad (Snow & Snow 1972). Schmid et al. (2010b) reported 13 ant species associated with flowers of this bromeliad, and one additional ant species (*Acromyrmex* sp.) was observed by us cutting *A. nudicaulis* flowers. Summing up all flower visitors recorded to date 66 species of floral visitors (17 species of hummingbirds) are known for *Ae. nudicaulis*. This is a high number, but taking into account the wide distribution of this bromeliad from Brazil to Mexico (Beutelspacher 1972), we regard our results on species richness still as an underestimation and assume that there might be many more visitor species - hummingbirds as well as insects - associated to this bromeliad.

Influence of habitat and latitudinal gradient on richness and spectrum of flower visitors

Species richness. Recent studies on species richness in ants on Santa Catarina Island (Rosumek et al. 2009; Schmid et al. 2010b) indicate that species richness of ants in restinga sites is lower than in forests sites. Consistently with those findings, we recorded a lower species richness of flower visitors within the restinga sites compared to the two forest habitats. The forest areas in SC and PR were similar in total species richness but differed considerably in species count within the visitor groups. We recorded an equal number of bird, bee and butterfly species in PR, whereas there was a high species richness of bees and butterflies and low richness of birds in SC. In contrast, we found no such differences between the two restinga habitats. These differences in richness between forest and restinga sites may be explained by differences in habitat heterogeneity (possibly influenced by latitudinal gradients) and also by the different ages of the habitats (Pianka 1966). Structurally complex habitats may provide more niches and diverse ways of exploiting the environmental resources and thus increase species diversity (Pianka 1966, Tews 2004). Also, when communities get old, they tend to become more diverse (Pianka 1966). At the study site in PR, the secondary forest is less disturbed than both forest and restinga habitats on Santa Catarina Island where, due to anthropogenic disturbance, the forests might be impoverished. Also, the secondary forest in PR is older, has higher trees, and the area covered with continuous forest is larger than the forest fragments on Santa Catarina Island, so it seems likely that the forest site in PR has a greater habitat heterogeneity due to a higher plant diversity (Bazzaz 1975) and might support an even more diverse floral visitor community than the habitats in SC, resulting in the higher species richness in floral visitors recorded here. This possibility is strengthened by the fact that the richness estimators for PR did not yet reach a plateau.

Species spectrum. Whereas the species richness was similar between the forest sites in SC and PR as well as between the two restinga sites on Santa Catarina Island, all sites differed in species composition of the floral visitor assemblage. Although there is an overlap in hummingbird species between SC and PR (*Th. glaucopis* and *Am. fimbriata)*, the spectrum of hummingbirds visiting *Ae. nudicaulis* in PR was broader including species not recorded in SC. This may be due to a better conservation status of that area. The similarity of PR and SC sites in bee species is zero, but there is overlap at the genus and family level (*Ceratina, Augochlora*, Apidae, Halictidae). Species of these genera have in common their elongate and slender body shape (body length < 10 mm) that allowed these small bees to crawl deep enough into the corolla to reach the nectar with their tongues. *Augochlora* bees, notwithstanding being classified as “short-tongued bees” based on structural characters rather than absolute tongue length (Michener 2000), have relatively long tongues (Video S8). Due to the morphology of *Ceratina* and *Augochlora* bees they can be expected to belong to the same functional group (Fenster et al. 2004). On Santa Catarina Island, we recorded both small and large to very large bees (ten out of 170 bee species, Steiner et al. 2010), but only small bees (five out of 119 bee species, Maia 2008) in the forest in PR. Although an evaluation of the melittofauna at Cachoeira Reserve (Maia 2008) showed that the larger bee visitors recorded in SC – *B. brasiliensis*, *E. annectans* and *X. brasilianorum* – also occurred there, they were not recorded as flower visitors. This suggests that flowers of *Ae. nudicaulis* might be less attractive to large bees in the forest in PR, maybe due to the low nectar standing crop resulting from the high visitation frequency of hummingbirds in this area. However, our results indicate an association of *Aechmea* flowers with the bee families Apidae and Halictidae at all sites.

The higher similarity between the studied habitats on Santa Catarina Island may be explained by the fact that these areas are closer to each other than with PR, which means that our finding of a higher proportion of shared species between these sites was to be expected.

Influence of habitat type on the frequency and diel pattern of flower visitors

The most striking difference between forest sites in PR and SC concerned the frequencies of hummingbirds and bees. The ranking of these taxa is reversed with hummingbirds being the most frequent visitors in PR, outnumbering bees but also insects as a whole. The situation in PR is consistent with what would be expected for a hummingbird-pollinated bromeliad. In fact, *Ae. nudicaulis* flowers have been classified as ornithophilous earlier (Araújo et al. 2004). A recent, more in-depth analysis of the pollination ecology, however, revealed a bimodal pollination system, with floral traits being intermediary between ornithophily and melittophily (Schmid et al. 2011), in which bees may also act as pollinators. Domination of the exploitation of the floral resources, namely nectar, by frequent visits of hummingbirds appears to be the more regular scenario for a well preserved, old, continuous forest site (Liebsch et al. 2007). Bees, on the other hand, would be expected to monopolise flowers as food resources only if hummingbirds occur in low abundance or forage mostly on other, more attractive floral resources on Santa Catarina Island. Unfortunately, quantitative assessments of hummingbird populations are not available for both areas.

Within the hummingbird assemblage observed in Paraná *An. nigricollis* dominated the floral resource nectar because of its distinct territorial behaviour. Perhaps due to their slightly greater size individuals of *An. nigricollis* (6.9 g, Snow & Snow 1972) were able to chase away the other hummingbirds (*Am. fimbriata:* 5.1 g, Weis-Fogh 1972; *Am. versicolor:* 4.1 g, Bech et al. 1997; *Th. glaucopis:* 5 g, Marini et al. 1997). This behaviour indicates that there is inter- and intraspecific competition among the hummingbirds. Birds with such a strong territorial behaviour that they stay close to one or a few *Ae. nudicaulis* plants are poor pollinators because they do not provide xenogamy which is required for seed set in this self-incompatible bromeliad (Schmid et al. 2011).

The much lower visitation frequencies recorded at the restinga habitats than at both forest habitats in PR and SC may result from the likewise lower species richness and the habitat-specific species composition of the dune habitats: about twice as many bee species have been recorded in forest than in restinga sites on Santa Catarina Island (unpublished data).

Wasps were observed as frequent visitors only in PR. This may be due to the fact that they only licked extrafloral nectar with their short tongues and they therefore did not compete with hummingbirds and bees in PR, which collected either floral nectar or pollen. In SC habitats, where ants (Schmid et al. 2010b), small bees and flies also exploited extrafloral nectar, *Aechmea* flowers may be less attractive to wasps. Additionally, most of the wasps observed by us were social hymenopterans and therefore tend to aggregate on food resources near the nest.

Whereas hummingbirds exploited all available open flowers of an inflorescence during their visits, as did the large bees of the genera *Xylocopa*, *Bombus*, and *Euglossa* to some extent, small bees and the heliconiid butterflies often visited only part of the open flowers. Depending on the availability of pollen, an augochlorine bee sometimes only needed to exploit one flower to complete her pollen load and then left the inflorescence. These examples show that visitors that need to make optimal use of floral resources and exploit flowers and inflorescences in an orderly sequence will have high values of visitation diligence and might act as better pollen vectors than visitors which left the inflorescences to return to the nest soon after exploiting one or few flowers.

The unimodal pattern of flower visitation with highest rates around noon was not strongly influenced by habitat type or geographic location. Our results show that the daily pattern of flower visitation is stronger influenced by bee than by hummingbird visits. In the forest of PR, the diel pattern of visitation is caused by the bees' higher activity later in the morning when the ambient temperature was adequate for foraging flights. In contrast, the hummingbird visits were evenly distributed along the day, because they are independent from ambient temperatures. Only in the morning hours, hummingbirds visit flowers in a slightly higher frequency than later the day. This may be because they need to regain energy lost during the night and because nectar secretion is higher in the morning. The pattern observed for *Ae. nudicaulis* in PR fits better to the results reported for other bromeliad studies made in São Paulo and Rio (Canela & Sazima 2003) than the pattern found in the SC habitats. There, overall visitation peaked around midday with pollen being depleted by bees in the early hours. The pattern of bee visitation almost exactly reflects this pattern. This is caused by the dominance of bees at the flowers.

Importance of bromeliads for the flower visitors and vice versa

The relationship between hummingbird and bromeliads is certainly mutualistic as the birds pollinate the plants and receive nectar as a reward. The same can be said for some of the associated bee species that can be pollinators of *Ae. nudicaulis* flowers (Schmid et al. 2011).

However, depending on the bee species (long-tongued *Euglossa* f. ex.) or the situation (pollen or nectar collection without moving between unrelated plants) they may also be nectar or pollen robbers. Especially in SC where bees dominated *Aechmea* flowers, pollen was rapidly harvested in the early morning hours, reducing the time frame for successful pollination to two or three hours only. Most of the other visitors exploit the floral resources pollen and floral or extrafloral nectar without contributing to pollination, acting therefore as pollen or nectar thieves (Inouye 1980). In heliconiid butterflies, for example, most of the pollen accumulated at the base of their proboscides is processed and digested extraorally soon after collection (Gilbert 1972; Boggs et al. 1981; O'Brien et al. 2003), and therefore the butterflies are only poor pollen vectors for *Ae. nudicaulis* (Schmid et al. 2011). Herbivores fed on floral tissue, sometimes destroying reproductive structures, as was reported for caterpillars of the genus *Strymon* (Robbins 2010; Schmid et al. 2010a). These herbivores have a negative effect because they clearly decrease the reproductive success of the infested plants.

Although *Ae. nudicaulis* plants are pollinated by only a small fraction of its visitors (Schmid et al. 2011), our results presented here clearly show that the flowers provide important, steady and diverse food sources (due to the extended flowering period) for a large group of animals but especially for hummingbirds, bees and butterflies. Flowers are available over a rather long period and therefore these plants support diverse assemblages of animals with different feeding habits and live history and sustain diversity in forest and restinga habitats on Santa Catarina Island as well as in the Cachoeira Reserve. In contrast to the expectation that *Ae. nudicaulis* flowers, due to its floral morphology, attract mainly hummingbirds, we showed that the flowers attract a much wider spectrum of animal species, mainly insects and arachnids. Our findings answer the question what attracts animals to the inflorescences and allows to classify them.

Online Supplementary Material

Figure S1. Comparison of species accumulation curves (species observed, sobs) of flower visitor species on *Aechmea nudicaulis.*

Video S2. Hummingbird *Aphantochroa cirrochloris* visiting flowers of *Aechmea nudicaulis* in urban area on Santa Catarina Island, southern Brazil.

Video S3. Hummingbird *Thalurania glaucopis* visiting *Aechmea nudicaulis* flowers in a secondary forest area on Santa Catarina Island, southern Brazil.

Videos S4. Orchid bee female (*Euglossa annectans*) visiting *Aechmea nudicaulis* in a secondary forest area on Santa Catarina Island, southern Brazil. After leaving the flower, the bee cleans its proboscis with the forelegs.

Video S5. Orchid bee male (*Euglossa annectans*) visiting *Aechmea nudicaulis* in a secondary forest area on Santa Catarina Island, southern Brazil.

Video S6. Carpenter bee (*Xylocopa artifex*) visiting a flower of *Aechmea nudicaulis* in a secondary forest area on Santa Catarina Island, southern Brazil. The bee is pushing its head inside the flower to reach the nectar.

Video S7. Bumble bee worker (*Bombus brasiliensis*) visiting *Aechmea nudicaulis* in a secondary forest area on Santa Catarina Island, southern Brazil.

Video S8. Augochlorine bee foraging for pollen on a flower of *Aechmea nudicaulis* in a secondary forest area on Santa Catarina Island, southern Brazil.

Table S9. Modified species list giving frequencies of floral visitors of *Aechmea nudicaulis* on species level.

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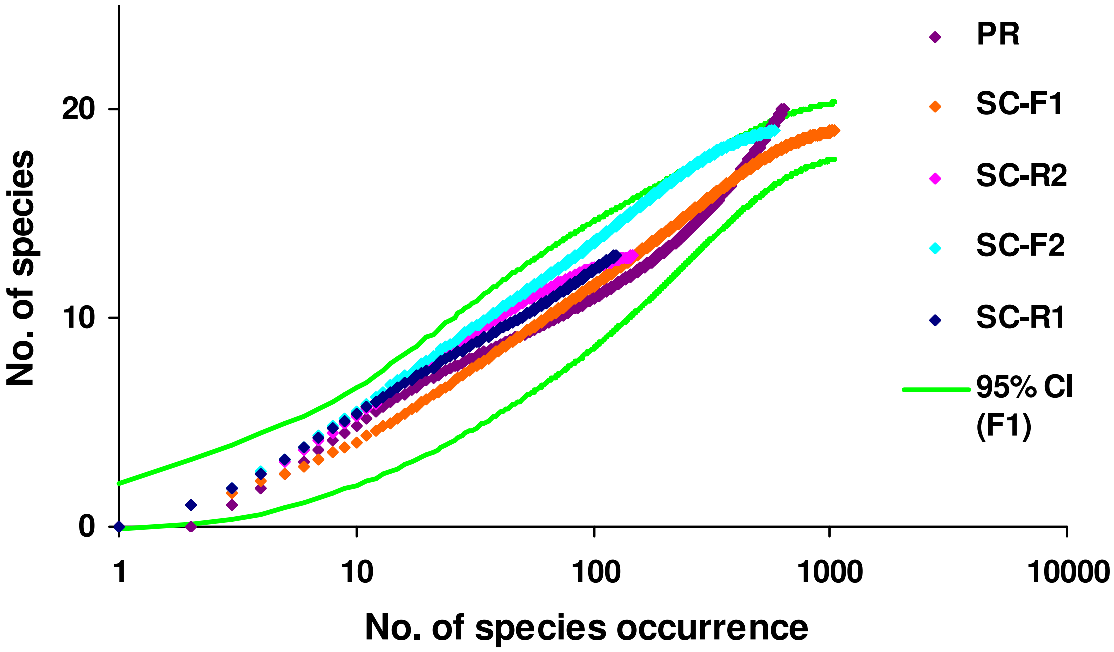
Table S8. Modified species list (Table 2) to give frequencies of floral visitors of *Aechmea nudicaulis* on species level. Unidentified specimens were either distributed over already existing taxa (according to their observed frequency proportions) or counted as one additional species. For the group Brachycera, neither could be reasonably assumed so this group was left out for the analyses.

	SC-F1	SC-F2	SC-R1	SC-R2	PR
Amazilia fimbriata	0	0	16	0	11
Amazilia versicolor	0	0	0	0	92
Anthracothorax nigricollis	0	0	0	0	183
Thalurania glaucopis	39	39	26	17	64
Coereba flaveola	0	0	8	0	2
Apis mellifera	0	0	0	0	1
Bombus brasiliensis	590	134	0	0	0
Ceratina (Crewella) sp.1	7	7	0	0	0
Ceratina (Crewella) sp.2	0	0	0	0	88
Euglossa annectans	56	56	0	14	0
Plebeia droryana	32	32	2	0	0
Meliponini	0	0	0	0	16
Trigona spinipes	0	0	21	0	0
Xylocopa brasilianorum	13	13	26	26	0
Xylocopa artifex	105	105	0	2	0
Augochlora (A.) sp.1	0	0	8	0	0
Augochlora (A.) sp.2	0	0	0	0	80
Augochlora (A.) sp.3	106	106	0	0	0
Augochlorella ephyra	0	0	0	49	0
Imara pallasia	27	27	1	0	0
Synpalamides phalaris	6	6	0	9	0
Geyeria decussata	3	3	0	7	0
Pieridae (*Phoebis* sp.)	3	3	2	0	3
Strymon ziba	3	3	9	5	0
Strymon sp.	0	0	0	0	1
Heliconius ethilla	36	36	1	0	19
Hesperiidae sp.1	0	0	0	3	0
Lepidoptera sp.1	0	0	0	0	1
Lepidoptera sp.2	0	0	0	4	0
Miridae sp. 1	2	2	0	0	0
Reduviidae sp.1	4	4	0	0	0
Curculionidae sp.1	5	5	0	0	1
Curculionidae sp.2	0	0	0	8	1
Curculionidae sp.3	0	0	0	0	2
Chrysomelidae sp.1	0	0	0	0	1
Salticidae sp.	1	1	2	2	1
Caelifera sp.	0	0	0	1	0
Vespidae sp.1	0	0	0	0	59
Vespidae sp.2	3	3	1	0	0


No. of species
20
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No. of species occurrence
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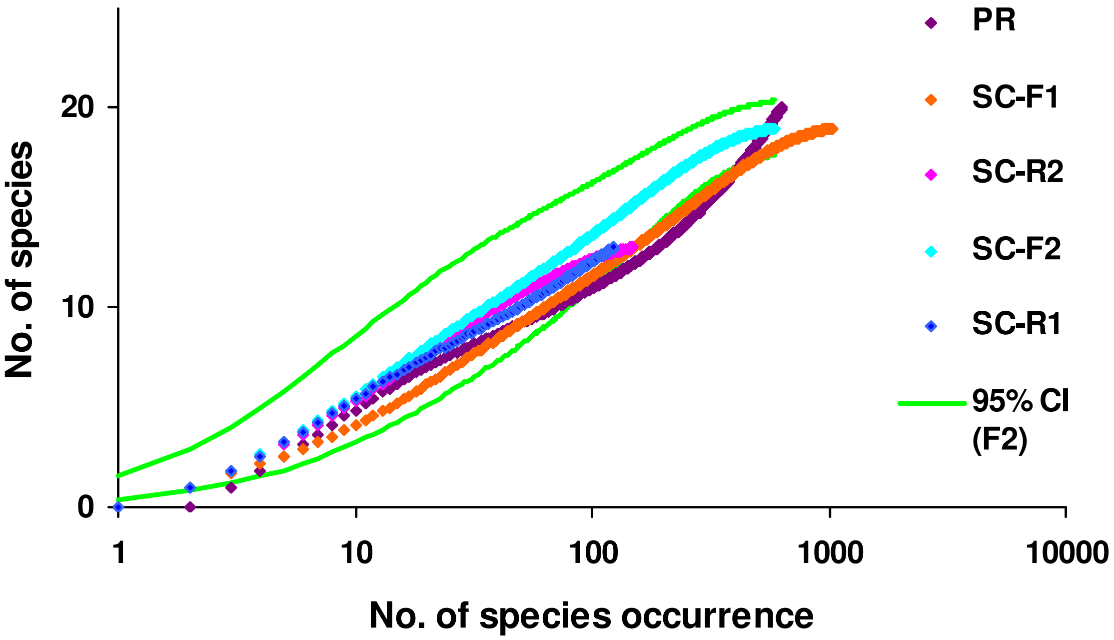
SC-F1
SC-R2
SC-F2
SC-R1
95% CI (F1)

A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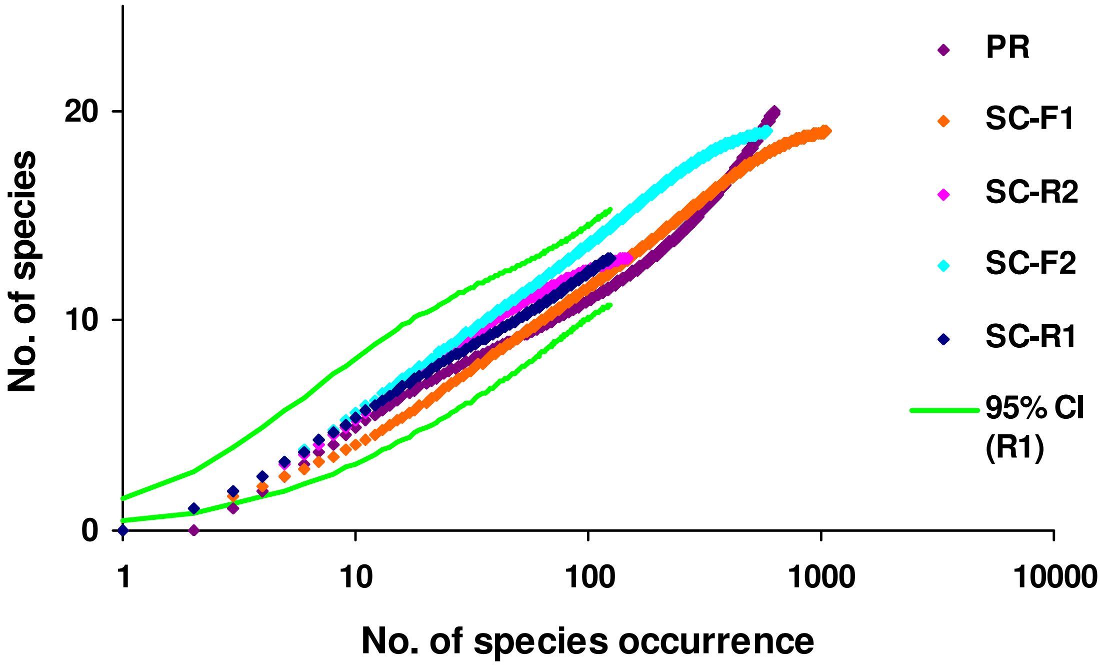
SC-R2
SC-F2
SC-R1
95% CI (F2)

B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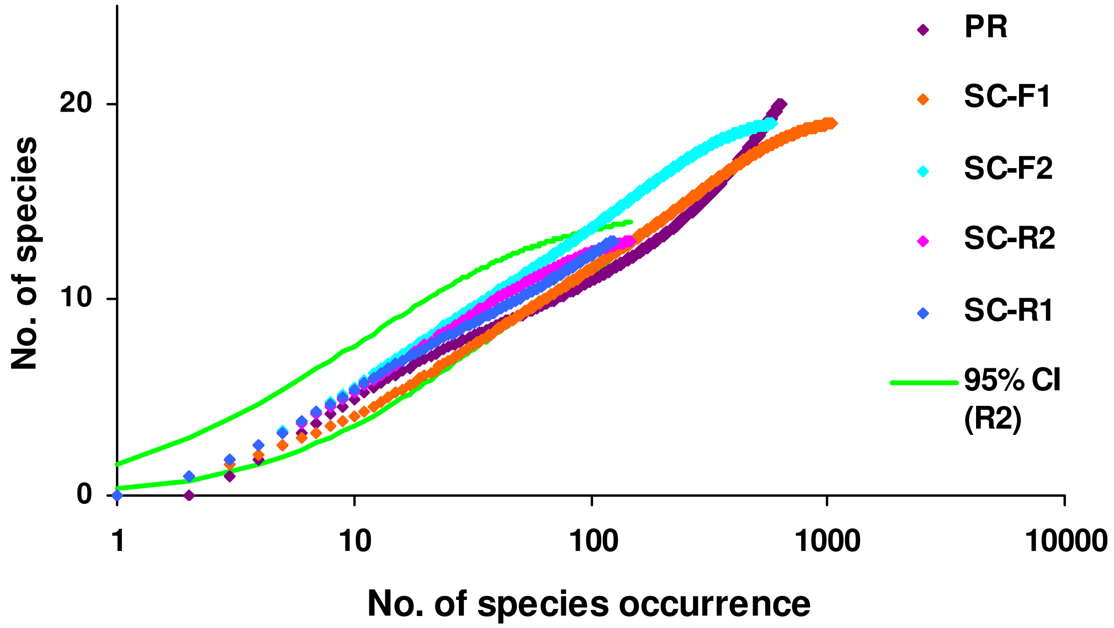
SC-R1
95% CI (R1)

C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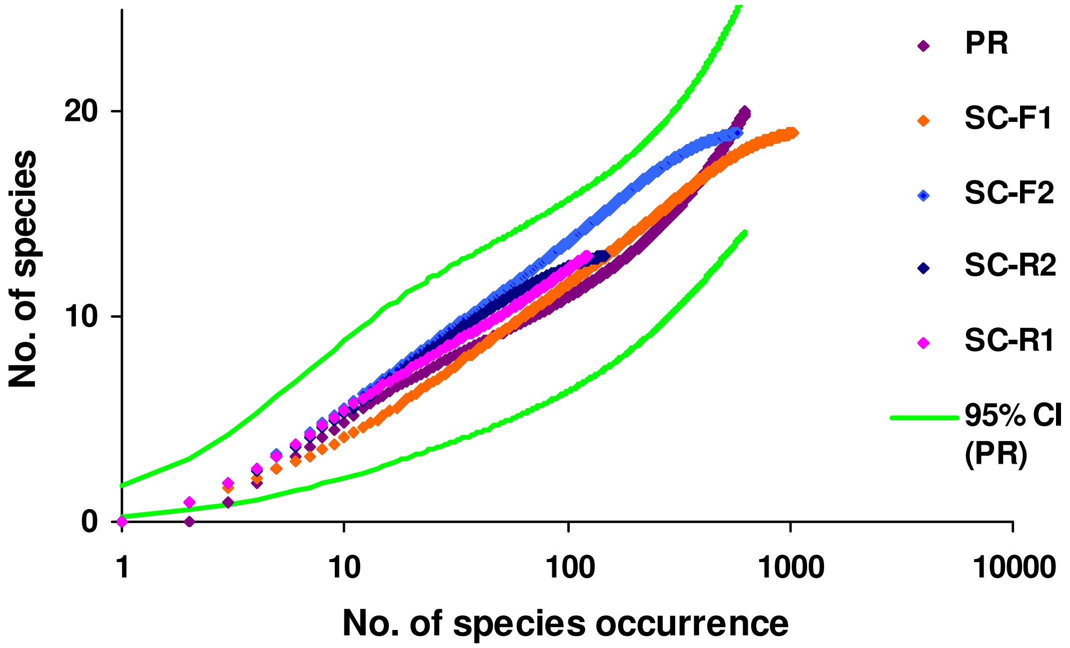
SC-F2
SC-R2
SC-R1
95% CI (R2)

D



E

Figure S1. Comparison of species accumulation curves (species observed, sobs) of flower visitor species on *Aechmea nudicaulis* on Santa Catarina Island and in Paraná, southern Brazil. Analysis is based on the data in Supplementary Table 1. For each of the four habitats SC-F1 (A), SC-F2 (B), SC-R1 (C), SC-R2 (D) and PR (E) the 95% confidence interval (CI) is shown.

Veröffentlichung 6: Schmid S, *Schmid VS, Zillikens A & J Steiner. Diversity of flower visitors and their role for pollination in the ornithophilous bromeliad Vriesea friburgensis in different habitats in southern Brazil. Eingereicht bei Ecotropica.*

Die Blütenbiologie von *Vriesea friburgensis* und ihre Blühperiode im Atlantischen Regenwald in Südbrasilien wurden untersucht. Außerdem wurden die Nektarproduktion und das Besucherspektrum analysiert. Letzteres wurde mit dem der Bromelie *Ae. nudicaulis* verglichen, da beide in denselben Gebieten vorkommen und somit denselben potentiellen Besuchern ausgesetzt sind. Aufgrund des stärker ornithophil ausgeprägten Syndroms der Blüten von *V. friburgensis* wurde die Hypothese aufgestellt, dass Bienen als Blütenbesucher eine untergeordnete und als Bestäuber keine Rolle spielen. *Vriesea friburgensis* blühte von Oktober bis Februar und schloss sich somit an die Blühperiode von *Ae. nudicaulis* an. Im Gegensatz zu dieser ist das reproduktive System von *V. friburgensis* teilweise autogam, wobei aber eine automatische Selbstbestäubung aufgrund der Blütenmorphologie (Stigma überragt stets die Antheren) ausgeschlossen ist. Die Blüten sind demnach von Besuchern als Pollenüberträgern abhängig; allerdings ist zur Bestäubung kein Fremdpollen nötig. Die mittlere Nektarkonzentration betrug 20,7%, der Nektar war reich an Saccharose. Eine Blüte produzierte im Mittel 73,9 µl Nektar pro Tag. Trotz des stark ausgeprägten ornithophilen Blütensyndroms wurden neben zwei Kolibriarten 28 weitere Arten von Insekten und Spinnen an den Blüten verzeichnet. Hierbei wurden alle Tiere, die mit den Blüten in Kontakt traten, als Blütenbesucher im weitesten Sinne definiert.

Veröffentlichung 6: Diversity of flower visitors and their role for pollination in the ornithophilous bromeliad *Vriesea friburgensis* in different habitats in southern Brazil

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Running title: Flower ecology of *Vriesea friburgensis*

Abstract. In order to analyze species richness of flower visitors of the bromeliad *V. friburgensis* and to relate the visitor spectrum to resource offer and differences in habitat, we studied its floral biology in two habitat types, dune vegetation and secondary Atlantic rain forest on Santa Catarina Island, southern Brazil. Flowering extends from October to February, the anthesis is diurnal. We found the reproductive system to be partially autogamous without the possibility of intra-floral selfing. Therefore, the bromeliad is pollinator-dependent, but the flowers do not need to be cross-pollinated. Mean nectar sugar concentration was 20.7% with a high sucrose proportion. Flowers secreted 73.9 µl nectar per day. Although the flowers show ornithophilous features like tubular and scentless flowers and higher energetic nectar in the morning hours, besides two hummingbird species we recorded 28 species of insects and spiders also being associated to the flowers of *V. friburgensis*. Species richness of flower visitors was higher in restinga than in secondary forest. In both habitat types hummingbirds and bees were the most frequent visitor groups. Whereas hummingbirds were the most frequent taxon visiting flowers in secondary forest followed by bees, it was just opposite in the restinga habitats. Because *V. friburgensis* is partially autogamous, small bees might also be pollinators, transferring self-pollen. Thus, there is redundancy in the pollination service provided by birds and bees, granting a high probability of successful reproduction in this bromeliad species.

Keywords: Bees; Bromeliaceae; ornithophily; flowering biology; hummingbird; nectar analysis; Santa Catarina Island; Southern Brazil; visitation frequency

INTRODUCTION

The neotropical plant family Bromeliaceae comprises epiphytic and terrestrial forms, many with large showy flowers (Benzing 2000). Bird pollination is the most frequent floral syndrome (Benzing 2000, Kessler & Krömer 2000), and hummingbirds appear to be effective pollinators (Benzing 2000). On the other hand, there are several reports of insects, e.g. bees and butterflies, visiting bromeliad flowers (Bernardello et al. 1991, Wendt et al. 2001, 2002, Canela & Sazima 2003, Schmid et al. 2011). In particular, many short-corolla bromeliads with otherwise typical ornithophilous syndrome are frequently visited by a high diversity of bees (Schmid et al. 2011).

Vriesea friburgensis var *paludosa* (Bromeliaceae: Bromelioideae) is endemic in Brazil and common in the coastal areas of the southern states Santa Catarina, Paraná and Rio Grande do Sul (Reitz 1983). Plants reproduce through clonal growth and via seeds, and each rosette produces only one inflorescence during its lifetime. Floral traits like tubular shape and color suggest the flowers to be adapted to hummingbird visitation (Faegri & van der Pijl 1971, Sick 1993). In fact, the two hummingbird species *Thalurania glaucopis* and *Amazilia fimbriata* have been reported as visitors to *V. friburgensis* flowers in the Atlantic forest in Paraná (Piacentini & Varassin 2007, Cestari 2009). Recently, Schmid et al. (2011) showed for the bromeliad species *Aechmea nudicaulis* that although the flowers exhibit an apparently ornithophilous syndrome, bees can also be regarded as important pollen vectors. The bromeliad *V. friburgensis* grows sympatrically with *Ae. nudicaulis* in both secondary forest and restinga on Santa Catarina Island. The long and tubular flowers of *V. friburgensis* show a more pronounced ornithophily than those of *Ae. nudicaulis*. Therefore, hummingbirds can be assumed to be the only visitors being able to access the nectar chambers. Although both bromeliad species grow in the same habitats and are exposed to the same visitor community, we hypothesize that (i) the visitor spectrum of *V. friburgensis* is poorer, especially in bee species, due to flower morphology, and (ii) that bees do not play a role in pollination.

Studying the species richness of bromeliad flower visitors and the diversity and nature of their animal–plant interactions, this study focused on recording the species spectrum and frequency of floral visitors of *V. friburgensis*. To understand what attracts visitors to the flowers, we (1) analyzed floral phenology and reproductive system, as well as the quality and timing of floral rewards. We further hypothesize that habitat has an influence on the species richness and diversity of floral visitors (Tews et al. 2004, Schmid et al., submitted), so we (2) compared the species spectrum of flower visitors to *V. friburgensis* between two habitat types, secondary forest and restinga (sand dune habitat). Finally, we (3) compared the floral visitor spectrum of *V. friburgensis* with that of the sympatrically growing bromeliad species *Ae. nudicaulis* to analyze the influence of the bromeliad species and different floral morphologies on species spectrum.

MATERIAL & METHODS

Study site. The study was conducted at Florianópolis on Santa Catarina Island, southern Brazil, during four flowering seasons of *V. friburgensis*, from December to February 2005/2006, 2006/2007, 2007/2008 and 2008/2009 each. Field work was carried out at three sites, differing in habitat type: dune vegetation at (1) Joaquina Beach and (2) Campeche Beach (27°37'37" S, 48°26'59" W) ('restinga'; Sampaio *et al.* 2002) and a hillside secondary forest area at (3) Santo Antônio de Lisboa (Zillikens *et al.* 2001; 27°30'26"S 48°30'28"W, hereafter Sto. Antônio). The distance between the forest and the restinga sites is 14 km. Time specification is given in standard time.

Flowering period and analysis of breeding system. Emergence and development of inflorescences were observed weekly from 24 August 2007 to 13 March 2008 at all study sites. Per site, all inflorescences visible along defined trails were counted. Three categories were defined: (i) "new": from first sight of new inflorescences still covered by bracts till bud stage; (ii) "open": with open flowers; and (iii) "withered": with only withered flowers and developing fruits. The ramification pattern and the arrangement and orientation of the flowers are shown in Grohme *et al.* (2007). Total number of flowers per inflorescence was counted for 17 inflorescences (ten from Sto. Antônio, seven from Joaquina). Additionally, the number of open flowers per day and inflorescence was recorded for seven inflorescences in Sto. Antônio throughout their whole flowering phase.

Open flowers per day were counted for 517 inflorescences in Joaquina on ten days while always walking along the same trail. Because inflorescences flower over a long period, several inflorescences were scored not only once, but were always counted as independent inflorescences. The number of days with open flowers for an inflorescence was analyzed for seven plants located in the miconietum in Sto. Antônio.

The breeding system was assessed with hand-pollination treatments on plants taken to the laboratory: autonomous self-pollination, manual self-pollination, and manual cross-pollination (n = 8 flowers each treatment) (Dafni 1992). Additionally, flowers in the field (n =

13 flowers of 12 plants) were marked as control for natural pollination. The 'Index of self-incompatibility' (ISI) and 'Index of automatic self-pollination' (IAS) were calculated according to Zapata & Arroyo (1978).

Analysis of nectar composition. Nectar samples were collected from 12 flowers of four plants at 2-hour intervals, from 05:00 h to 15:00 h, and sugar composition and concentration were analyzed (for detailed methods see Schmid *et al.* 2011). The total volume was measured by removing the remaining nectar with 5 to 20 µl micropipettes (Assistent, Germany), and adding to this volume the previously collected 2 µl. The concentrations of the three sugars every two hours were Spearman-correlated and the differences between the three sugars were analyzed using the Wilcoxon Signed-Rank Test computed with JMP 8.01. The method to calculate the total energetic value of the nectar was the same as used for nectar of *A. nudicaulis* flowers by Schmid *et al.* (2011). For the 05:00 h sample nectar production is not exactly known because it could not be measured in the still closed flowers. Sugar concentration was not measured after 15:00 h because almost no nectar was present at that time.

Flower visitor spectrum, visitation frequency, and foraging behavior. In total, 80 inflorescences were observed in situ at Joaquina Beach and Sto. Antônio either directly or with binoculars (314 total observation hours) to record taxa and frequency of flower visitors. As flowers open only one single day, one inflorescence observed on different days was counted as two independent observation events because the flowers were different in position and number. We recorded floral visitors in the widest sense, so all animals touching a flower were counted as visitors. For every approaching visitor we recorded species, time of arrival and leaving, and the kind of reward collected. Voucher specimens were deposited in the collection of Josefina Steiner, LANUFSC, Federal University of Santa Catarina, Brazil.

RESULTS

Flowering biology and breeding system of V. friburgensis. Inflorescences started to appear in October. The flowering period extended from November to February. Bud stage as well as flowering period and presence of infructescences were synchronous at the three sites (Fig. 1). The zygomorph flowers were tubular and without scent. Anthers and stigma protruded from the corolla. Flowers began to open between 01:00 h and 02:00 h (Fig. 2). The stigma came out first, followed by the anthers. Around 05:00 h the flower was fully open, but the anthers dehisced not until 06:00 h with sunset. Effective depth of flowers (the depth of the corolla which a visitor has to overcome to reach the nectar) was 29 mm (median; Q1 = 28, Q3 = 32; range 24.8 – 37.5 mm; n = 33 flowers, 10 plants). Flower entrance width measured 5 mm (median; Q1 = 4.5, Q3 = 5, range 3.9 – 6.1 mm; n = 33 flowers, 10 plants).

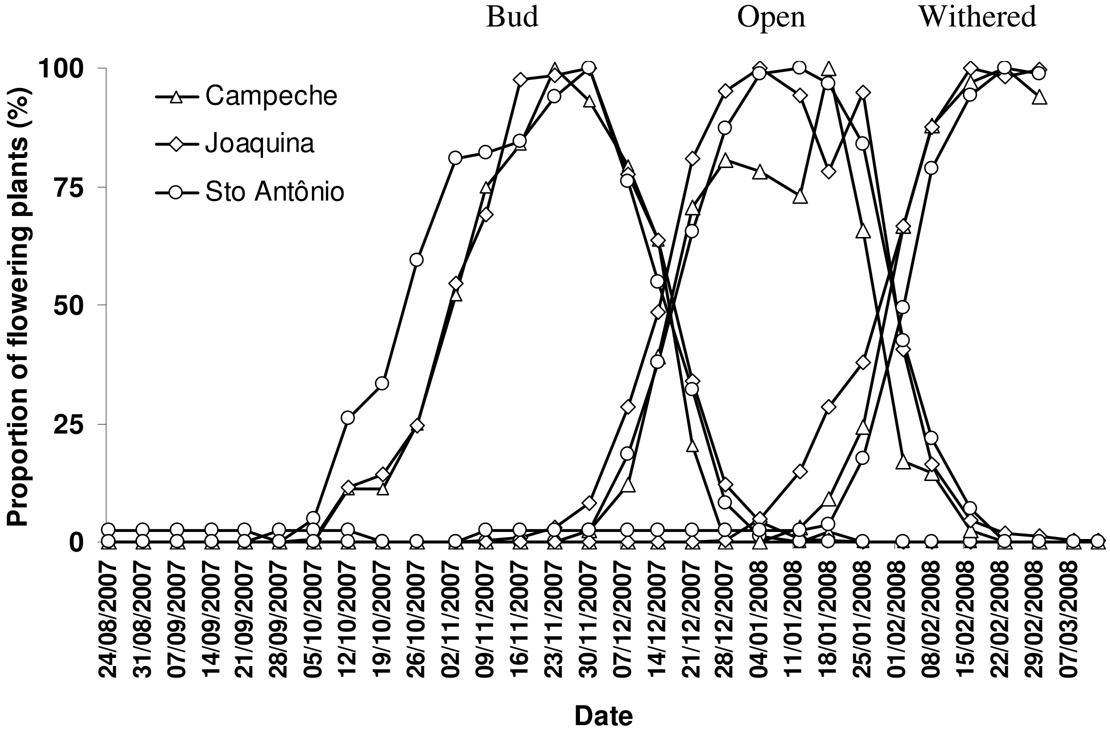

FIG. 1. Period of *Vriesea friburgensis* inflorescences with buds, open and withered flowers at the three sites Sto. Antônio (circle), Campeche Beach (triangle), and Joaquina Beach (diamond) on Santa Catarina Island, Brazil.

Due to the fact that the numbers of flowers per inflorescence in the two habitats Joaquina and Sto. Antônio were not normally distributed and also significantly different (Mann-Whitney U test, JMP 8.01, SAS Institute Inc, 2009; $\chi^2 = 11.6667$, d.f. = 1, p = 0.0006), medians for Joaquina and Sto. Antônio were computed separately. Number of flowers per inflorescence was 54 in Sto. Antônio (median; Q1 = 46.5, Q3 = 72.5; range 26 – 53) and 113 in Joaquina (median; Q1 = 85.75, Q3 = 152.75; range 80 – 172). Number of open flowers per day was 1 (median; Q1 = 1; Q3 = 2; range 0 – 7; n = 517 infl, 10 days). Between zero and two open flowers per day were most frequently observed (see Fig. 3). Mean number of days with open flowers was 42 ± 10.3 (mean ± SD).

FIG. 2. Process of flower opening in *Vriesea friburgensis* on Santa Catarina Island, Brazil.

TABLE 1. Seed numbers resulting from pollination experiments with *Vriesea friburgensis* on Santa Catarina Island, southern Brazil, to analyze the breeding system.

Pollination treatment	N	Mean no. of seeds	Range	SD
manual cross-pollination	8	188.1	0-316	94.5
manual self-pollination	8	115.4	0-242	106.0
autonomous selfing	8	0	-	0
natural pollination	13	361.62	237-442	71.79

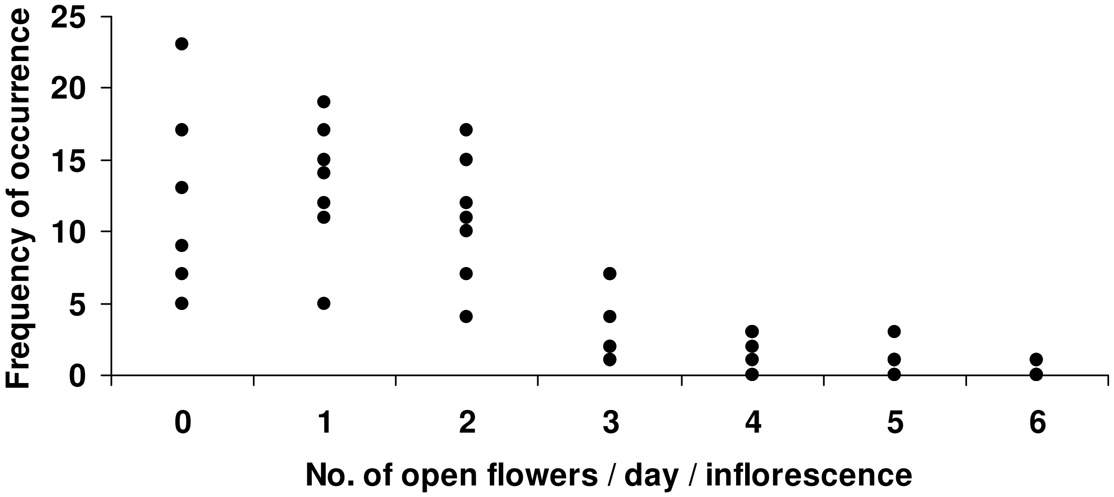

FIG. 3. Scatterplot of the frequency distribution of the number of open flowers per inflorescence and day of *Vriesea friburgensis* (n = 7 plants) in Sto. Antônio, Santa Catarina Island, Southern Brazil. Each data point represents the number of days with the corresponding number of open flowers (0 – 6 flowers open per day) during the flowering period for one inflorescence.

There was seed set in the two treatments manual self- and manual cross-pollination as well as in naturally pollinated flowers (Table 1). The ISI resulted in 0.61 (n = 16) and the IAS was 0 (n = 16). Induction of seed set, tested with manually cross-pollinated flowers, was possible during the whole phase of anthesis (from flower opening till closure, see Fig. 4).

Mean number of seeds per successfully developed fruit after natural pollination was 361.6 ± 71.8 and the percentage of undeveloped fruits in the field under natural conditions was 62.2% ± 16.7 of which 26.1% ± 15.6 were found to be parasitized by eurytomid wasps.

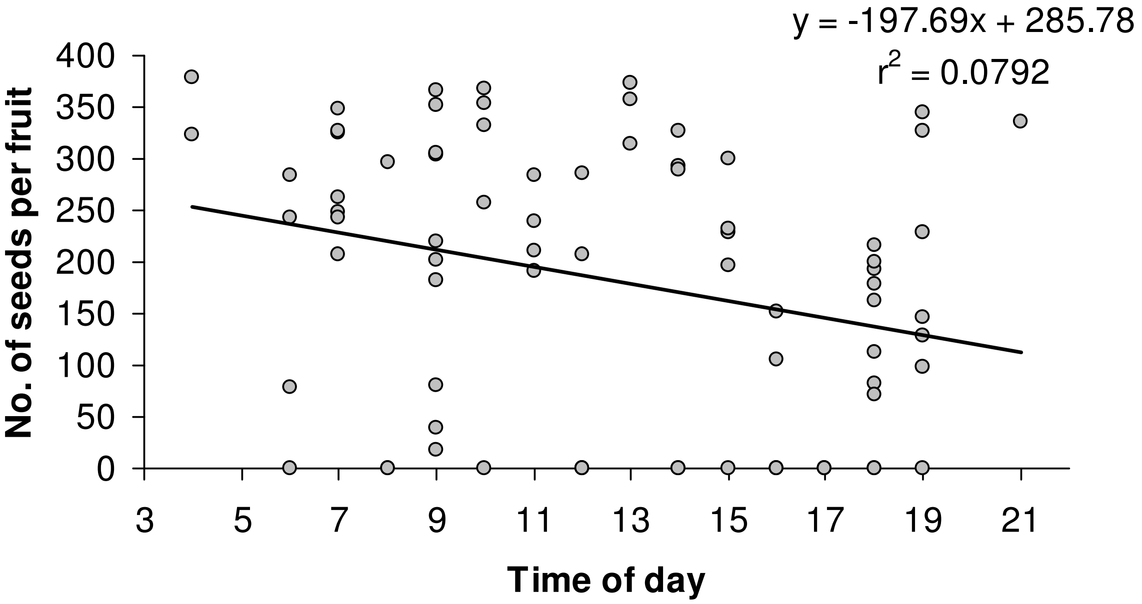

FIG. 4. Temporal pattern of the possibility of ovule fertilization in flowers of *Vriesea friburgensis*, Santa Catarina Island, southern Brazil.

Nectar analyses. The mean nectar volume per flower and hour was 6.4 µl ± 5.3 (SD). The production of nectar was not evenly distributed over anthesis and peaked at 11:00 (Fig. 5). Mean sugar concentration was 19.5% (w/w, SD = 6.8); highest concentration was measured at 05:00 h (Fig. 6). Sucrose secretion was higher than that of the hexoses, and most of the time the nectar was sucrose-dominant (Fig. 7). Mean overall sucrose-hexose ratio [S/(F+G)] was 1.65 ± 0.62 (mean, SD). Mean sucrose proportion per hour decreased during anthesis from 71.4% (05:00 h) to 43.1% (15:00 h). Mean total sugar secreted per flower and day was 14565.7 µg = 14.5 mg ± 0.9 mg (SD). The trend in sugar production was more similar to nectar concentration than to nectar volume; most sugar was secreted until 09:00 h. Concentration of fructose and glucose were significantly correlated with each other (Spearman correlation, Table 2) and fructose values were significantly higher than those of glucose (Wilcoxon Signed-Rank Test Statistic 1173.000, $p < 0.0001$). Sucrose concentration was not significantly correlated with concentration of both hexoses (Table 2).

Mean energy value per flower was 218.3 J ± 104.3 (SD). Multiplication by the mean number of flowers per inflorescence (Sto. Antônio: 44; Joaquina: 113) resulted in a mean caloric nectar value per inflorescence of 9.6 kJ for Sto. Antônio and 24.7 kJ for Joaquina.

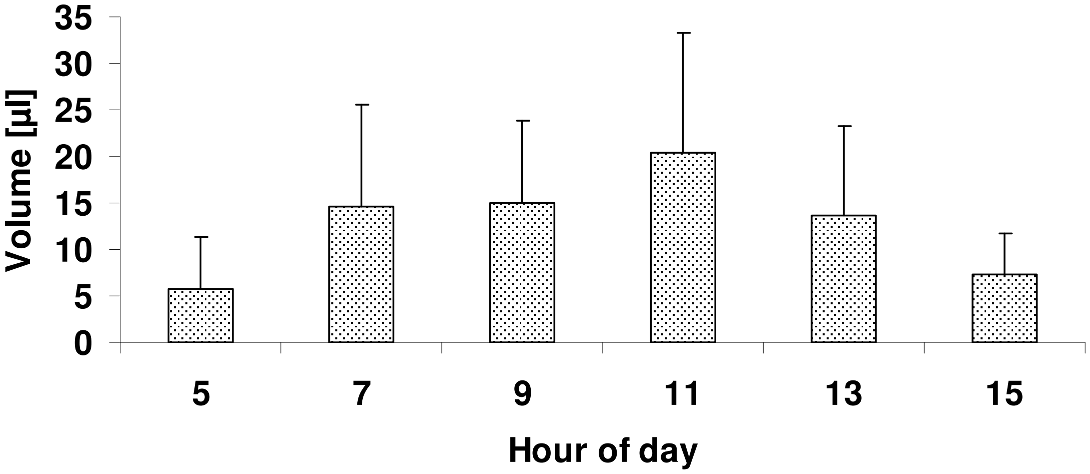

FIG. 5. Diel pattern of nectar volume produced every two hours per flower of *Vriesea friburgensis*, Santa Catarina Island, southern Brazil.

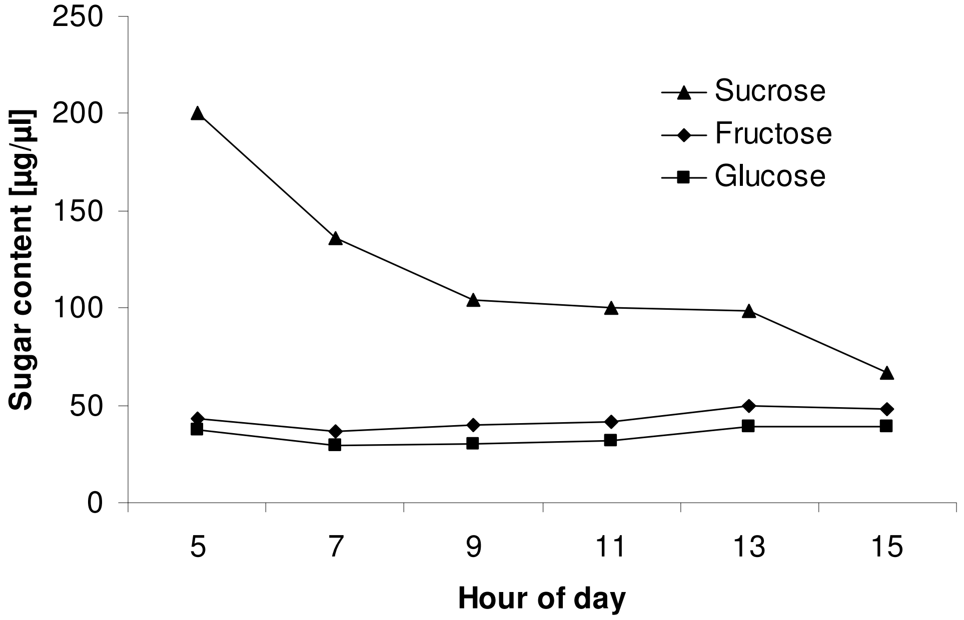

FIG. 6. Diel pattern of nectar sugar content in *Vriesea friburgensis* nectar: Sucrose concentration was high in the first hour of secretion and decreased continuously during the day.

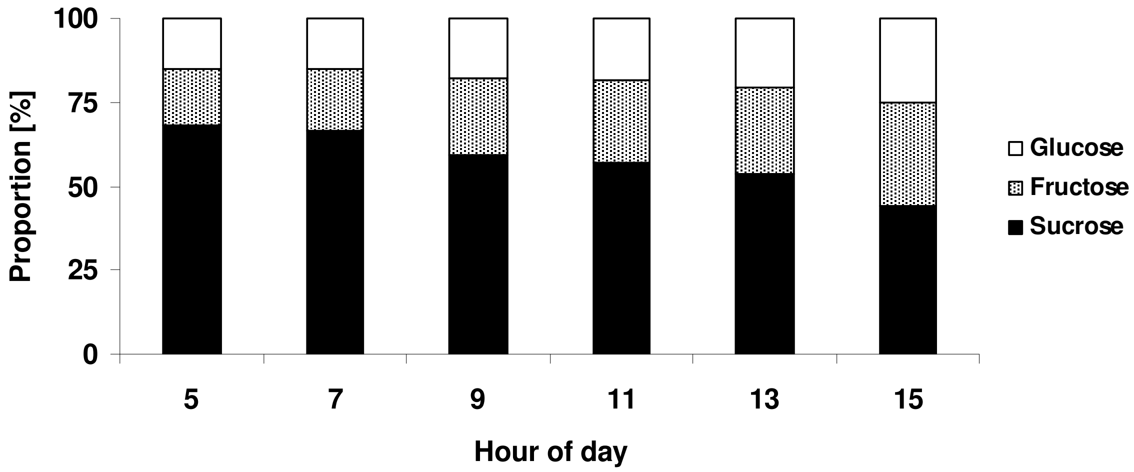

FIG. 7. Diel pattern of the proportion of the three sugars sucrose, fructose, and hexose in nectar of *Vriesea friburgensis*, Santa Catarina Island, southern Brazil.

TABLE 2. Spearman correlations between pairs of sugars contained in floral nectar of *V. friburgensis* on Santa Catarina Island, southern Brazil. Bonferroni correction yields a significance level of alpha = 0.05/18 = 0.0028. Significant p values are designated by an asterisk (*). G: glucose, F: fructose, S: sucrose.

	F – S		G – S		G – F	
Hour of day	Spearman ρ	P	Spearman ρ	P	Spearman ρ	P
05:00	0.2909	0.3855	0.1727	0.6115	0.9455	<0.0001*
07:00	0.1727	0.6115	0.1636	0.6307	0.9636	<0.0001*
09:00	0.3636	0.2453	0.0559	0.8629	0.8531	0.0004*
11:00	-0.2168	0.4986	-0.3007	0.3423	0.9091	<0.0001*
13:00	0.1469	0.6488	-0.1329	0.6806	0.9021	<0.0001*
15:00	-0.0061*	0.9867	-0.1394	0.7009	0.9515	<0.0001*

Flower visitor species spectrum, frequency, and foraging behavior. We recorded a total of 30 species as visitors, in the widest sense, to flowers of *V. friburgensis* (Table 3). The restinga habitat had a higher species richness (21 species, n = 1073 visitors) than the secondary forest (14 species, n= 304 visitors). Secondary forest and restinga sites shared 6 species. One species, the hummingbird *Eupetomena macroura*, was only observed attending flowers in an urban area. In both habitats the most frequent visitors were hummingbirds and bees. Whereas hummingbirds were the most frequent taxon visiting flowers of *V. friburgensis* in secondary forest followed by bees, it was the opposite on flowers in the restinga habitats (Fig. 8).

Hummingbirds inserted their bills into open flowers and touched the anthers and stigmas while hovering in front of the flowers (Fig. 9A-B, Video S1). One individual of the bee species *Xylocopa brasilianorum* was observed piercing the corolla tube to reach the nectar chamber (Fig. 9C). *Trigona spinipes, Ceratina* sp. and *Augochlorini* collected pollen with their forelegs while hanging upside down on the anthers. They then stored the pollen in corbiculae (Fig. 9D) or the ventral abdominal and hind leg scopae (Fig. 9D, F, H). Individuals of these bees were slender enough to enter the flowers to collect nectar, too (Fig. 9G). *Trigona spinipes* individuals also sometimes gnawed holes into the corolla base when the tube was too narrow for them (Fig. 9E). Occasionally, the augochlorine bees touched the stigma while foraging for pollen. One individual was observed expanding a regurgitated nectar droplet between hypostomal fossa (Engel 2000) and the proximal section of the proboscis (cardines and hypopharynx) (Krenn *et al.* 2005) after the visit (Fig. 9I). Beetles (Fig. 9J) and cockroaches (Fig. 9K) were observed on the inflorescences feeding on open and withered flowers. Flower mites gathered on the anthers to feed on pollen (Fig. 9L). Vespid wasps (Fig. 9M) were observed walking on the inflorescences, one individual tried to enter an open flower (Fig. 9N). Ants were not recorded systematically but they were observed regularly on and in flowers (Fig. 9O), preying on other animals and collecting nectar inside the flowers. Jumping spiders (Fig. 9P) and praying mantids (Fig. 9Q) lurked at the inflorescences for potential prey, for example ants (Fig. 9P).

TABLE 3. Species spectrum and frequency and collected resource of flower visitors of *Vriesea friburgensis* on Santa Catarina Island, southern Brazil. N = number of visits observed in field experiments are given in parentheses. Visitation aims: n = nectar, p = pollen, o = oviposition, h = herbivory, pr = predator. Because bees of the taxon Augochlorini could not be identified to species during flower visits, frequency and number of visits are given as sum and x in the table show the species occurrence in the habitats.

Taxon	**N**	**Visitation frequency (N)**		**Resources**
		SC Island		
		forest	restinga	
Trochilidae	**15.0 (207)**	**60.9 (185)**	**2.1 (22)**	**n**
Amazilia fimbriata	**10.3 (142)**	46.7 (142)	-	n
Thalurania glaucopis	**4.7 (65)**	14.1 (43)	2.1 (22)	n
*Eupetomena macroura**				n
Apoidea	**81.4 (1121)**	**37.2 (113)**	**93.9 (1008)**	**p + n**
Apidae	**11.0 (151)**	2.0 (6)	13.5 (145)	
Apis mellifera	**3.3 (45)**	-	4.2 (45)	p

Taxon	**N**	**Visitation frequency (N)**		**Resources**
Ceratina (Crewella) sp.1	**0.4 (5)**	1.6 (5)	-	p + n
Plebeia droryana	**0.1 (1)**	0.3 (1)	-	
Trigona spinipes	**6.9 (95)**	-	8.9 (95)	p + n
Xylocopa brasilianorum	**0.4 (5)**	-	0.5 (5)	p + n
Halictidae	**70.4 (969)**	35.2 (107)	80.3 (862)	p + n
Augochlora (A.) amphitrite		-	x	-
Augochlora (A.) sp.2		x	x	-
Augochlora (A.) sp.3		x	-	-
Augochlorella ephyra		x	x	-
Augochloropsis cfr. *cleopatra*		-	x	-
Augochloropsis cfr. *patens*		-	x	-
Dialictus cfr. *opacus*		-	x	-
Thectochlora hamata		-	x	-
Apoidea n.i.	**(1)**	(6)	(1)	
Lepidoptera	**-**	**x (1)**	**-**	**n + o**
Lepidoptera n.i.*		x (1)	-	n
*Strymon serapio**	**-**	x	-	n + o
Coleoptera	**0.7 (9)**	**-**	**0.8 (9)**	**h**
Araneae	**0.5 (7)**	**0.7 (2)**	**0.5 (5)**	**pr**
Araneae n.i.		(1)		pr
Thomisidae n.i.*			x	pr
Salticidae n.i.		(1)	(5)	pr
Diptera	**1.3 (18)**	**0.7 (2)**	**1.5 (16)**	**-**
Diptera n.i.		(2)	(16)	-
Saltatoria	**0.1 (2)**	**-**	**0.2 (2)**	**pr**
Saltatoria n.i.				pr
Mantidae	**0.1 (1)**	**-**	**0.1 (1)**	**pr**
Mantidae n.i.	(1)		(1)	pr
Vespidae	**0.8 (11)**	**(1)**	**1.0 (10)**	**pr + n**
Vespidae sp.1		**-**	**x**	**pr + n**
Vespidae sp.2		**-**	**x**	**pr + n**
Eurytoma sp.1	**0.4 (6)**	**0.3 (1)**	**0.5 (5)**	**o**
Blattaria	**0.1 (1)**	**0.3 (1)**	**-**	**h**
Blattaria sp.1	**-**	**x (1)**		**h**
Blattaria sp.2	**-**		**x**	**h**

* Individuals were observed as visitors but not during regular observation sessions so there is no frequency available.

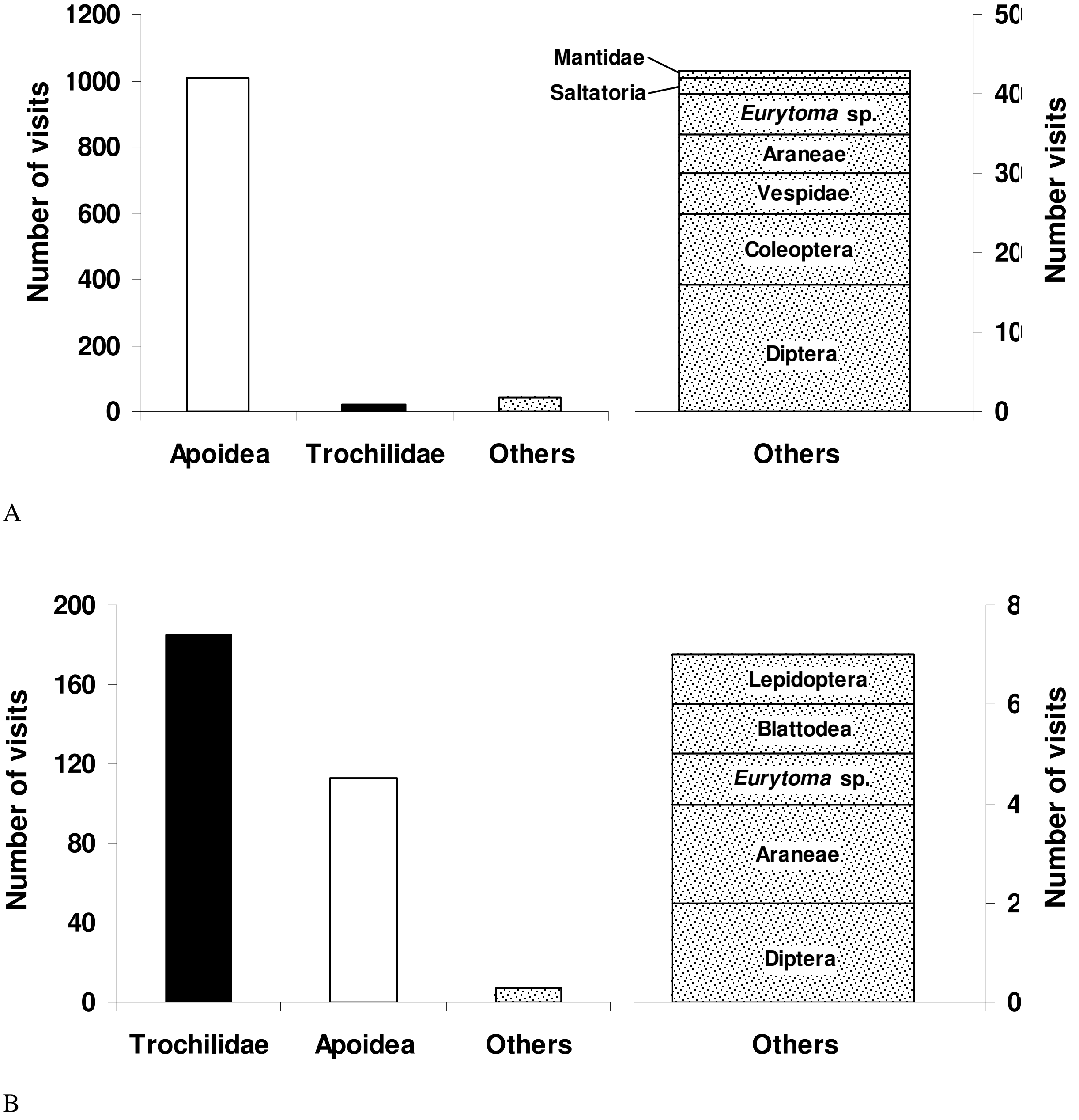

FIG. 8. Visitation frequency of flower visitor groups on *Vriesea friburgensis* flowers in restinga (A) and secondary forest (B) habitat on Santa Catarina Island, southern Brazil. On the right hand side, the detailed composition of the "others" fraction is shown as stacked column.

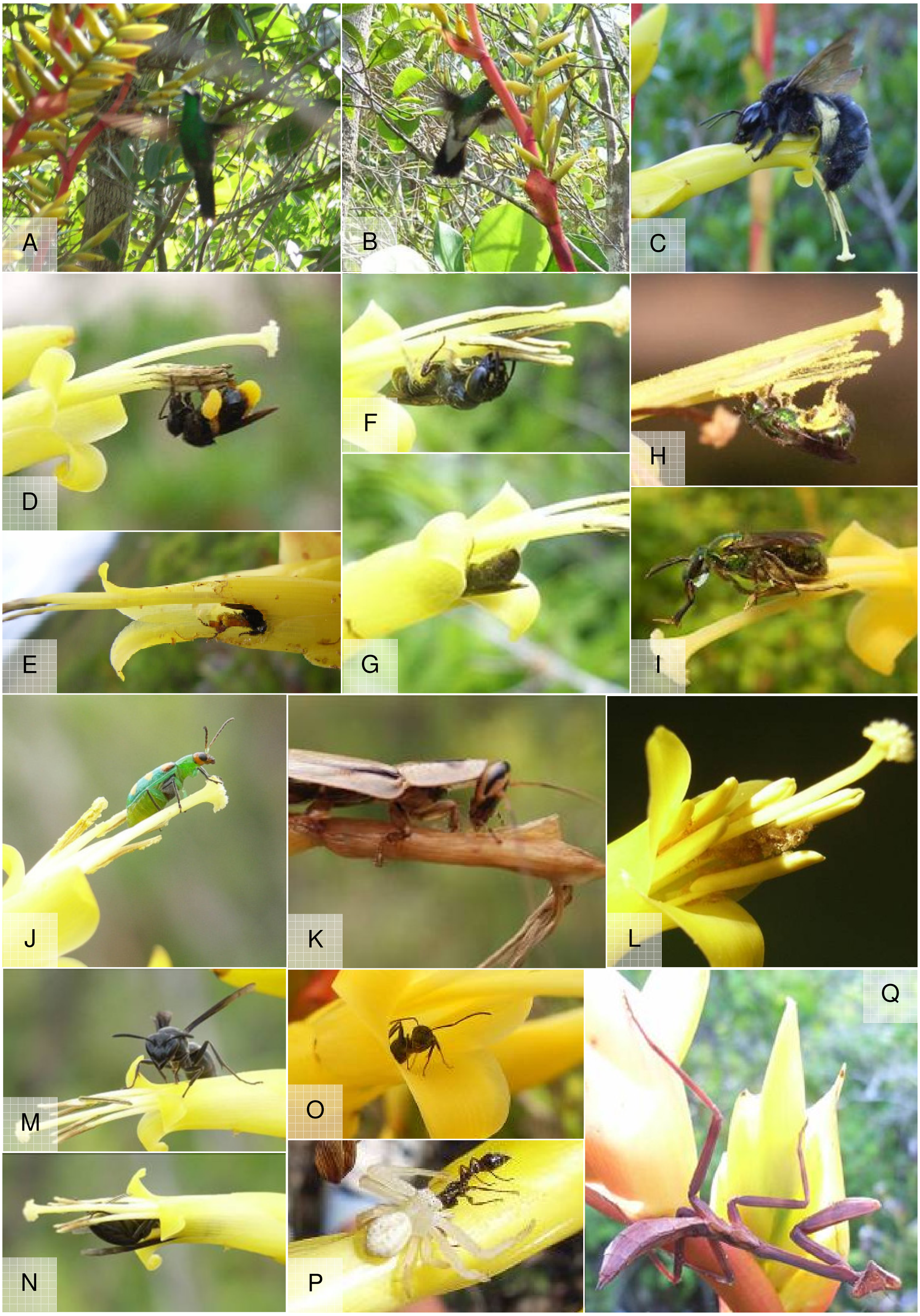
A
B
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FIG. 9. Visitors on flowers of *Vriesea friburgensis* on Santa Catarina Island, southern Brazil. *Thalurania glaucopis* (A) and *Amazilia fimbriata* (B) hovering in front of flowers. *Xylocopa brasilianorum* acting as nectar robber (C). *Trigona spinipes* collecting pollen (D) and robbing nectar (E). *Ceratina* female collecting pollen at the anthers (F) and entering the flower to collect the nectar (G). Pollen-collecting augochlorine bee (H) and an individual sitting on the stigma while "dehydrating" a nectar droplet (I). Chrysomelid beetle sitting on the stigma (J). Cockroach feeding on the withered petals (K). Flower mites sitting on the anthers (L). Vespid wasp on the flower in search for nectar (M-N). *Camponotus* worker ant guarding the flower entrance (O). Crab spider (Thomisidae) feeding on a *Pseudomyrmex gracilis* worker ant (P). Preying mantis sitting on inflorescence (Q).

The temporal pattern of nectar production and concentration throughout anthesis of *V. friburgensis* was similar to *Ae. nudicaulis* but the lower concentration and the higher amount of nectar secreted per hour in *V. friburgensis* flowers is better adapted to attract hummingbirds. Overall, nectar production features like high volume and sucrose content in the early morning hours as well as sucrose-dominant nectar seem to be adapted to attract mainly hummingbirds (Bernardello *et al.* 1991), and the flower morphology (long tubular flowers, anthers and stigma exposed, stigma slightly in front to touch the bird's forehead first) matches with the morphology of hummingbirds with straight and rather short bills. Large bees of genera like *Bombus* and *Xylocopa* or long-tongued bees of the genus *Euglossa* are able to drink large quantities of floral nectar and they can reach the nectar even in flowers with moderately ornithophilous features. These bees were reported as visitors of *Ae. nudicaulis* (Schmid *et al.* 2011) and *Ae. lindenii* (Dorneles 2006) but they cannot exploit, at least not in a legitimate way, *V. friburgensis* flowers. Therefore more nectar is available for the hummingbirds. This makes visits to *Vriesea* flowers more profitable for the birds than visits to *Aechmea* flowers.

The flower visitors of Vriesea friburgensis. In contrast to the clearly ornithophilous floral syndrome, we found a highly diverse visitor assemblage comprising many arthropods, especially insects. Also, we found a good concordance in the visitor spectrum of *V. friburgensis* and *Ae. nudicaulis*, recently studied in the same habitats (Schmid *et al.* 2011). The two hummingbird species that occur in secondary forest and restinga on Santa Catarina Island, *T. glaucopis* and *Am. fimbriata*, forage on both bromeliad species, and also in the bee spectrum there is an overlap in visitor taxa, namely several genera in the Apidae and Halictidae. This clearly shows that these two bromeliad species partly share the same visitor species, and, flowering one after another, support the local hummingbird and bee fauna over an extended period.

Contrary to the findings on *A. nudicaulis* (Schmid *et al.* 2011) and for ants in restinga habitats (Rosumek *et al.* 2008; Schmid *et al.* 2010 b), the species richness of flower visitors of *Vriesea* was higher in the restinga than in the secondary forest. Not only more species but also a higher diversity of taxonomic groups was observed. In restinga, flowers were mainly visited by bees, most of them augochlorine sweat bees. There, visitation frequency of hummingbirds was much lower than in secondary forest. This allowed higher bee visitation frequency, because nectar standing crop was not depleted by hummingbirds and thus was available for these bees which are small enough to crawl into the flowers. Also, much of the pollen remained inside the anthers where it could easily be harvested by bees.

Pollination biology of V. friburgensis vs. Ae. nudicaulis. In contrast to *Ae. nudicaulis* which is self-incompatible and therefore depending on outcrossing via pollinators (Schmid *et al.* 2011), the flowers of *V. friburgensis* are self-compatible but herkogamous because of spatial separation of stamina and stigma. So, pollen is not automatically transferred to the stigma and intra-floral selfing without an animal pollen vector is not possible. Consequently, pollination can take place when hummingbirds, forced to insert their bills deep into the corolla, touch the stigma with their foreheads dusted with pollen (see Video S1 and S2). Pollination by bees would have to occur in a different way. Pollen gathered by augochlorine and small carpenter bees is stored in the scopae of the hind legs and the abdomen, and is not, as in the apine bees, mixed with nectar to stick together as compact pellets. In fact, the rather loosely packed pollen may be transferred to the stigma while the bee is climbing over it. In *Ae. nudicaulis* only visitors that bring pollen from genetically different plants and have long (as in hummingbirds, *Bombus* spp. or *Xylocopa* spp.) but not too long (as in euglossines or butterflies) tongues or beaks, respectively, have a good chance of pollinating the flowers while drinking nectar. In contrast, on flowers of *V. friburgensis* even small bees with short tongues (Augochlorini and *Ceratina*) only engaged in pollen collection are capable of pollen transfer. The first scenario is much more likely for those flower visitors that perform a trap-lining behavior to satisfy their needs for a high and regular nectar uptake, such as hummingbirds and large bees. The second scenario, however, requires only pollen gathering bees. Bee species of the taxa Augochlorini and Ceratinini are among the most species rich and abundant taxa (Steiner *et al.* 2010). They occur all over the Neotropics, are abundant in forest as well as restinga, natural as well as modified habitats, and are active almost all year round (multivoltine) (Michener 2000). Both Augochlorini and Ceratinini are similar in their ecotype and therefore belong to the same functional group (Fenster *et al.* 2004). It appears that *V. friburgensis* uses both strategies – xenogamy and autogamy – to ensure successful pollination. Xenogamy is favored by forcing hummingbirds to perform trap-lining by offering, on average, only one open flower per plant so that pollen from unrelated plants is transferred with almost every flower visit. On the other side, autogamy is performed by a species rich group of generalist bee species characterized by a distinct mode of pollen collection and storing. Our results suggest that in places where the hummingbirds are rare visitors or even absent, bees can fill the gap and pollinate the flowers. Even if, from the plant's point of view, xenogamy is more desirable than autogamy, the second strategy eliminates the risk of a total failure of pollination in the absence of the legitimate pollinators. The high seed set per flower observed indicates that, at present, natural pollination is sufficient to assure the persistence of this bromeliad in the two habitats studied, even though the loss of seeds due to parasitation by *Eurytoma* wasps (Grohme *et al.* 2007) was high.

Although we did not measure individual pollinator effectiveness the short-billed hummingbirds occurring on Santa Catarina Island are supposedly the most effective pollen vectors of *V. friburgensis*. Somewhat unexpectedly for this bromeliad species with an even more pronounced ornithophilous syndrome than seen in *Ae. nudicaulis* (and other species of *Aechmea*), bees were also crucial part of the visitor assemblage, and not just pollen and nectar thieves. To what extend these bees provide pollination service has to be further investigated in carefully executed pollination experiments. In any case, our study points to the high importance of *V. friburgensis* flowers as food resource for the local bee fauna.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY VIDEO S1. The hummingbird *Amazilia fimbriata* visiting flowers of *Vriesea friburgensis* on Santa Catarina Island, southern Brazil.

SUPPLEMENTARY VIDEO S2. The hummingbird *Thalurania glaucopis* visiting a flower of *Vriesea friburgensis* on Santa Catarina Island, southern Brazil.

SUPPLEMENTARY VIDEO S3. Augochlorine bee collecting pollen at a *Vriesea friburgensis* flower on Santa Catarina Island, southern Brazil.

SUPPLEMENTARY VIDEO S4. Augochlorine bee entering a flower of *Vriesea friburgensis* in search for nectar in secondary forest habitat on Santa Catarina Island, southern Brazil. The bee is chased away by a worker ant guarding the flower.

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4 Diskussion

Beide Bromelienarten sind abhängig von Pollen übertragenden Blütenbesuchern. Während *Ae. nudicaulis* selbststeril ist und nur durch Fremdpollen bestäubt werden kann, ist bei *V. friburgensis* auch autogame Bestäubung möglich. Beide sind mit einem großen Spektrum von Blütenbesuchern assoziiert. Dies ähnelt der hohen Diversität der mit den Phytotelmen und den terrestrischen Mikrohabitaten innerhalb der Bromelienrosetten assoziierten Tiere (Wittman 2000; Frank & Lounibos 2008) und bestätigt die Rolle von Bromelien als Mikrokosmos in Bezug auf Diversität und Interaktionen der assoziierten Fauna.

Wie aufgrund der vorwiegend ornithophilen Blütenmerkmale erwartet, waren Kolibris die effizientesten Bestäuber. Überraschenderweise sind aber auch Bienen in der Lage, die Blüten beider Bromelienarten zu bestäuben. Das Blütensyndrom ist im Fall von *Ae. nudicaulis* nicht rein ornithophil sondern bimodal, da Kolibris und große Bienen ähnlich effektiv im Bestäuben der Blüten waren, wenn man die unterschiedlichen Besucherhäufigkeiten mit berücksichtigt. Die Blüten von *V. friburgensis* zeigten ein stärker ausgeprägtes ornithophiles Syndrom. Dennoch leisten hier die kleinen Bienen aus den Taxa Augochlorini und *Ceratina* möglicherweise auch einen Beitrag zur Samenproduktion, da sie während des Pollensammelns an den Antheren auch mit dem Stigma in Kontakt kommen und dabei Eigenpollen übertragen können. Beide Bromelienarten verfügen durch ihr Spektrum an möglichen Pollenüberträgern über eine gewisse Flexibilität in Bezug auf die Bestäubung und erreichen so eine Absicherung der Reproduktion. Das wiederum ermöglicht den Bromelien eine weite Verbreitung in verschiedenen Habitaten, da sie nicht an eine einzige Art von Bestäubern gebunden sind. Dies ist von Bedeutung, da ein deutlicher Einfluss des Habitattyps auf das Blütenbesucherspektrum beobachtet wurde.

Unter dem in meiner Arbeit weit gefassten Begriff „Blütenbesucher“ fallen auch solche Arten, die mit der Bestäubung zwar nichts zu tun haben, aber dennoch einen Einfluss auf den Fortpflanzungserfolg der untersuchten Bromelien ausüben. Hierzu gehört zum einen die von uns erstmalig an *V. friburgensis* gefundene Erzwespenart der Gattung *Eurytoma*, deren Larven die Blüten im Knospenstadium sterilisieren, so dass die Samenproduktion durch den Ausfall eines großen Teils der Blüten erheblich geschmälert wird. Die Assoziation dieser Wespenart mit *V. friburgensis* ist vermutlich hochspezifisch, denn vergleichbare Vorkommnisse wurden an anderen syntopischen Bromelienarten nicht beobachtet. Die Anpassung des Lebenszyklus an die Blühperiode der Bromelie lässt auf eine enge Wirt-

Parasit-Beziehung schließen. Somit handelt es sich hier um eine neu dokumentierte artspezifische Bromelien-Insekten-Interaktion.

Die Herbivorie, oder genauer die Frugivorie der Bromelienfrüchte durch Lycaeniden-Raupen war zwar schon länger bekannt, die von uns dokumentierten Assoziationen der drei *Strymon*-Arten zu *Aechmea*- und *Vriesea-Arten*, sowie die Befunde zu Lebenszyklus, Färbung und Verhalten waren bislang unbekannt. Die direkte Entwicklung der Raupen ohne Diapause lässt auf einen multivoltinen Zyklus (mehrere Generationen) der *Strymon*-Arten schließen. Damit verbunden ist vermutlich ein Wirtswechsel, da die Blühphasen der Bromelien eine artspezifisch unterschiedliche, klar definierte Saisonalität aufweisen. Die für *S. ziba* und *S. oreala*, beides Schädlinge in Ananaskulturen, entdeckten parasitären Wespen könnten möglicherweise zur Bekämpfung der Larven auf den Ananasfeldern genutzt werden, wenn es gelingt, den Lebenszyklus der Wespen aufzuklären und sie künstlich aufzuziehen.

Für die Besucher des an der Basis des Sepalen-„Dorns“ sezernierten extrafloralen Nektars konnte weder eine eindeutig positive noch eine negative Auswirkung auf die Fortpflanzung der *Aechmea*-Arten nachgewiesen werden. Unsere Beobachtungen der Ameisen an den Infloreszenzen von Bromelien bestätigen die Hypothese, dass das Hauptziel der zahlreichen Ameisen die Ausbeutung des extrafloralen Nektars ist. Gegen eine bloß „neutrale“ Rolle der durch den extrafloralen Nektar angelockten Ameisen spricht allerdings, dass die Nektar- und Pollen-fressenden Blütenmilben in der Natur selten so überhand nahmen wie unter ameisenfreien Laborbedingungen. Obwohl bislang unbewiesen, ist doch anzunehmen, dass die Ameisen den Bromelien von Nutzen sind, indem sie eine gewisse Kontrolle über die Milbenpopulation, eine unvermeidliche Konsequenz der Bestäubung durch Kolibris, ausüben. Dadurch würde der negative Einfluss der Milben durch den nicht zu unterschätzenden Nektar- und Pollenraub auf den Reproduktionserfolg der Bromelien gemildert.

5 Zusammenfassung - Resumo

Zusammenfassung

Im Rahmen des Projektes „Interne Dynamik des Regenwaldes: spezifische Tier-Pflanze-Interaktionen“ des deutsch-brasilianische Kooperationsprogramms „Mata Atlantica“ habe ich exemplarisch an zwei Modellorganismen, den Bromelienarten *Aechmea nudicaulis* und *Vriesea friburgensis*, deren Blüten- und Bestäubungsbiologie sowie für ihre Fortpflanzung relevante Tier-Pflanze-Interaktionen studiert. Im Wesentlichen sollte geklärt werden, welches reproduktive System bei den beiden Bromelien vorliegt und wer die effektiven Bestäuber dieser Pflanzen sind. Dazu wurden die Blühperiode und phänotypische Blütenmerkmale untersucht sowie die Zusammensetzung und das tageszeitliche Sekretionsmuster des Nektars analysiert. Für beide Bromelien wurden umfassende Besucherspektren aufgenommen. Die Beobachtungen wurden in zwei unterschiedlichen Habitaten und an unterschiedlichen Breitengraden durchgeführt.

Das Blütensyndrom ist im Fall von *Aechmea nudicaulis* bimodal, bei *Vriesea friburgensis* zeigen die Blüten stärker ornithophile Merkmale. Beide Bromelienarten sind abhängig von Pollen übertragenden Blütenbesuchern, da sie sich nicht autonom bestäuben können. Kolibris waren, wie es die Blütensyndrome erwarten ließen, die effizientesten Bestäuber. Unerwarteterweise konnten aber auch Bienen die Blüten beider Bromelienarten bestäuben. Beide Arten sind mit einem großen Spektrum von Blütenbesuchern assoziiert. Dies ähnelt der hohen Diversität der mit den Phytotelmen und den terrestrischen Mikrohabitaten innerhalb der Bromelienrosetten assoziierten Tiere und bestätigt deren Rolle als Mikrokosmos. Die vorliegende Studie hat zudem einen eindeutigen Einfluss des Habitattyps auf das Blütenbesucherspektrum nachgewiesen.

Zwischen den Blütenständen von Bromelien und den blütenbesuchenden Tieren sowie auch unter den Letztgenannten gibt es ein breites Spektrum an Interaktionen, das weit über die Bestäubung der Blüten hinausgeht, so zum Beispiel Pollen- und Nektarraub, Herbivorie, Prädation, Parasitismus und Frugivorie. Einige dieser Interaktionen, die für den Fortpflanzungserfolg der Bromelien von Bedeutung sind wurden, z. T. erstmalig beschrieben und analysiert. Es sind dies die Sterilisierung von Blütenknospen durch herbivore Larven von Erzwespen, die Vernichtung von Samen- bzw. Samenanlagen durch herbivore Schmetterlingsraupen und deren Parasitierung durch Wespen, sowie die Anlockung von Ameisen zu *Aechmea*-Blüten, vermutlich mit der Folge einer Reduktion der Population von Blütenmilben.

Für die untersuchten Bromelienarten scheint die sexuelle Reproduktion durch die Bestäuberaktivitäten gesichert zu sein. Die Bestäubung durch mehrere funktionale Gruppen stellt eine Redundanz der Ökosystemfunktion Bestäubung durch Diversität der Bestäuberumgebung dar. Bromelien bilden einen wichtigen Knotenpunkt im Beziehungsnetz des Atlantischen Regenwalds, da sie einen großen und vielfältigen Nutzen für die Tierwelt haben und deshalb ein wichtiger Faktor zum Erhalt der Biodiversität dieses Lebensraums sind.

Resumo

Dentro do programa alemão-brasileiro "Mata Atlântica", no projeto "Dinâmica interna de florestas pluviais: interações específicas entre animais e plantas", eu estudei a biologia floral, a polinização, e outras interações entre animais e plantas relevantes para a reprodução de dois modelos, as espécies de bromélias *Aechmea nudicaulis* e *Vriesea friburgensis*. O objetivo deste trabalho foi esclarecer o sistema reprodutivo das duas espécies de bromélias e identificar os polinizadores efetivos das mesmas. O período de floração e a fenologia floral foram examinados assim como a composição e o padrão diário da secreção do néctar. O espectro completo dos visitantes florais foi registrado para as duas bromélias. As observações foram realizadas em habitats com vegetação de latitude diferente.

A síndrome de ornitofilia de *Aechmea nudicaulis* é bimodal, em quanto *Vriesea friburgensis* tem flores com características ornitofilas mais fortes. Ambas as espécies dependem de visitantes florais para transmitir o pólen visto que a polinização das flores não é autônoma. Conforme a síndrome floral os beija-flores foram os polinizadores mais eficientes. Inesperadamente, abelhas também foram capazes de polinizar as flores de ambas bromélias. *Aechmea nudicaulis* e *V. friburgensis* são associados a uma grande diversidade de visitantes florais. Isto parece estar relacionado com a grande diversidade de animais associados aos fitotélmatas e aos micro-habitats terrestres dentro das rosetas, o que confirma a importância das bromélias como microcosmo. Além disto este estudo demonstrou a influência evidente do tipo de habitat ao espectro dos visitantes florais.

Existe um grande espectro de associações entre inflorescências de *Aechmea* e *Vriesea* e os visitantes florais, bem como entre os próprios visitantes que vai muito além da polinização como exemplos o roubo de pólen e nectar, herbivoria, predação, parasitismo e frugivoria. Interações imporantes para a reprodução eficaz das bromélias foram descritas e analisadas; sendo que algumas pela primeira vez. A esterilização de botões por larvas de vespas do grupo Chalcidoidea, a destruição de sementes e ovulos por lagartas herbívoras e o parasitismo deles por larvas de vespas, assim como a atração de formigas às inflorecências de *Aechmea*, provavelmente com a conseqüência de uma reduzir as populações dos ácaros florais são exemplos de interações não descritas até o momento.

Para as espécies de bromélias estudadas, a reprodução sexuada parece estar seguro pela atividade dos polinizadores. A polinização feita por vários grupos funcionais é uma redundância nesta função do ecossistema através da diversidade do ambiente de polinizadores. Bromélias formam um nó importante dentro da rede de relações dos organismos da Mata Atlântica pela grande e variada utilidade para a fauna e por isso são elementos importantes para a preservação da biodiversidade deste habitat.

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Erklärung

Ich erkläre hiermit, dass ich die zur Promotion eingereichte Arbeit mit dem Titel: „Die Rolle von Bienen und anderen Bestäubern in der Reproduktionsbiologie der Bromelien *Aechmea nudicaulis* und *Vriesea friburgensis* im Atlantischen Regenwald Südbrasiliens“ selbstständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

Tübingen, den 11.08.2010

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