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Morcegos frugívoros como facilitadores da regeneração natural em áreas degradadas

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Orientadora: Dra. Sandra Bos Mikich

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São José do Rio Preto
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“(...) mais um exemplo mostrando como plantas e animais, tão afastados uns dos outros na escala da natureza, são conectados por uma teia de relações complexas.”

DARWIN, CHARLES (1859) - *The Origin of Species*

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RESUMO

Morcegos frugívoros são bons dispersores de sementes e por utilizarem o olfato como forma de identificação de frutos maduros, foi proposta uma ferramenta de restauração que atrai estes animais com o uso de óleos essenciais de frutos zoocóricos, como forma de aumentar a chuva de sementes em áreas degradadas. Esta nova ferramenta de restauração está em constante desenvolvimento e aqui (i) foi analisada e comparada a composição dos óleos essenciais dos frutos preferidos de *Artibeus*, *Carollia* e *Sturnira*, com outros frutos consumidos e não consumidos por estes morcegos; (ii) utilizamos a análise cromatográfica combinada com experimentos em cativeiro de dupla escolha para analisar o papel de diferentes compostos orgânicos voláteis (VOCs) na atração destes filostomídeos; (iii) foi feita uma revisão bibliográfica da dieta dos morcegos do Velho Mundo para analisar a congruência entre a dieta frugívora de Pteropodidae com uma nova proposta zoogeográfica, e por fim, (iv) foram analisados gêneros de Pteropodidae que poderiam ser utilizados, com base na diversidade de sua dieta e preferência por frutos, em programas de restauração como Phyllostomidae estão no Novo Mundo. Os nossos resultados mostram que os óleos essenciais de frutos consumidos pelos filostomídeos frugívoros compartilham compostos, mas os preferidos podem ser identificados por compostos-chave. Além disso, esses animais podem identificar dois tipos diferentes de VOCs - monoterpenos e sesquiterpenos - cada um com uma função diferente na comunicação morcego-fruto. A dieta de raposas-voadoras mostrou-se diversa e congruente com os reinos zoogeográficos, tendo *Ficus* como o gênero de fruto mais consumido. Os pteropodídeos *Cynopterus*, *Pteropus* e *Rousettus* foram identificados como modelos para a adoção da técnica de restauração nos continentes tropicais onde os filostomídeos não estão presentes. Dessa forma, a presente tese traz contribuições importantes à ecologia de morcegos, à ecologia química e à ecologia da restauração.

Palavras-chave: Chiroptera, dispersão de sementes, *Ficus*, olfato, Phyllostomidae, Pteropodidae

ABSTRACT

Fruit bats are good seed dispersers and since they use odorific clues to locate and select mature fruits, they have been attracted to degraded areas with essential oils extracted from their preferred fruits in order to increase seed rain. This novel restoration tool is under constant development and here we (i) compared the composition of the essential oils of the preferred fruits of *Artibeus*, *Carollia* and *Sturnira*, with other fruits consumed and ignored by these bats; (ii) used chromatographic analysis combined with double-choice captive experiments to analyze the role of different volatile organic compounds (VOCs) in the attraction of these phyllostomids; (iii) conducted a bibliographical review of the diet of Old World bats to analyze the congruence between the frugivorous diet of Pteropodidae with a new zoogeographic proposal, and finally (iv) identified genera of Pteropodidae that could be used, based on the diversity of their diet and fruit preference, in restoration programs like Phyllostomidae are in the New World. We discovered that the essential oils of fruits consumed by the frugivorous phyllostomids share compounds, but the preferred ones can be identified by key compounds. In addition, these animals revealed that they can identify two different types of VOCs - monoterpenes and sesquiterpenes - each with a different function in bat-fruit communication. The diet of flying foxes was found to be diverse and congruent with the zoogeographic realms, having *Ficus* as the most consumed fruit genus. Additionally, the pteropodids *Cynopterus*, *Pteropus* and *Rousettus* were identified as potential models for the adoption of the restoration tool in tropical continents where phyllostomids are not present. Thus, in all, the present thesis brings important contribution to the ecology of bats, chemical ecology and restoration ecology.

Key words: Chiroptera, *Ficus*, olfaction, Phyllostomidae, Pteropodidae, seed dispersal

SUMÁRIO

INTRODUÇÃO GERAL

A interação entre animais e plantas é uma das grandes responsáveis pela biodiversidade do planeta. Relações mutualísticas entre angiospermas e animais são de grande importância ecológica e evolutiva nos ecossistemas tropicais e subtropicais (BASCOMPTE; JORDANO, 2007; FLEMING; KRESS, 2013). O transporte de pólen ou sementes por um vetor animal, traz benefícios nutricionais para o vetor e mobilidade para a planta, que consegue reproduzir e germinar suas sementes em um local adequado (WANG; SMITH, 2002). Apesar de poder ser realizada por diversos grupos, a dispersão de sementes, diferente da polinização, é um processo mais relacionado aos vertebrados, principalmente pelo tamanho dos propágulos transportados (FLEMING; KRESS, 2013). Aves e morcegos se destacam neste cenário por sua capacidade de voo e de cruzar grandes distâncias, além da diversidade e quantidade de espécies de plantas consumidas, fazem destes grupos bons dispersores (ARTEAGA; AGUIRRE; MOYA, 2006; GONZALES et al., 2009).

Morcegos frugívoros

Como vetores de dispersão, os morcegos frugívoros, foco deste estudo, se diferenciam das aves por defecarem principalmente em voo e não empoleirados, aumentando assim a distância das sementes da planta-mãe e a probabilidade de sua chegada em ambientes degradados (MUSCARELLA; FLEMING, 2007).

A frugivoria em Chiroptera evoluiu independentemente em duas famílias: Pteropodidae e Phyllostomidae. Os primeiros são conhecidos como morcegos do Velho Mundo e são distribuídos em parte da África, Ásia, Oceania e várias de suas ilhas do Pacífico (IUCN, 2016; SIMMONS, 2005). Com quase 190 espécies reconhecidas e 42 gêneros, os megaquirópteros podem variar de 42 a 200 mm de comprimento do antebraço, com seus maiores exemplares em gêneros como *Pteropus* Brisson, 1762 e *Acerodon* Jourdan, 1837 (ALMEIDA et al., 2011; NOWAK, 1999; SIMMONS, 2005), que chegam a cerca de 2 m de envergadura (NOWAK, 1999; STIER; MILDENSTEIN, 2005). Todas suas espécies são herbívoras, sendo que 36 dos seus 42 gêneros são considerados frugívoros (FLEMING; KRESS, 2013). Os outros seis são considerados nectarívoros, ainda que consumam frutos eventualmente (e.g. Law 2001).

Já os filostomídeos se distribuem pela América do Sul, Central, com alguns gêneros chegando até o sul dos Estados Unidos (GARDNER, 2007). Os tamanhos variam de 47 a 130 mm de comprimento total e antebraço de 26 a 106 mm (NOWAK, 1999) e, por comparação com o grupo anterior, são conhecidos como microquirópteros. Ao contrário dos frugívoros do Velho Mundo, os filostomídeos não são todos fitófagos; apenas cerca de 20 dos seus 44 gêneros alimentam-se de frutos (FLEMING; KRESS, 2013; mas ver MONTEIRO; NOGUEIRA, 2011).

Além do hábito alimentar, outro fator em comum destaca-se entre estes frugívoros: a sua acuidade olfativa. O olfato é definido por Wackermannová et al. (2016) como o mediador da percepção de compostos químicos voláteis, trazendo informações do ambiente ao receptor. Na interação morcego-fruto, o olfato é utilizado para interpretar as características do alimento, que se apresentam em uma combinação de compostos orgânicos voláteis (VOCs), que são produzidos e emitidos no início do seu desenvolvimento e diminuem ao longo do processo de maturação (DUDAREVA et al., 2013). Os VOCs ou óleos essenciais, são considerados aleloquímicos sinomônios, por serem compostos relacionados à comunicação interespecífica e a trazerem benefícios tanto para o emissor, quanto para o receptor (HICK; LUSZNIAK; PICKETT, 1999; WHITTAKER; FEENY, 1971).

Os representantes pteropodídeos possuem melhor visão que seus parentes filostomídeos e em contrapartida não ecolocalizam (com exceção de *Rousettus* Gray, 1821 que possui ecolocalização por meio de 'clicks' produzido pela língua), mas ambos possuem o olfato como peça chave para identificação e seleção de seus frutos maduros (FLEMING, 1988; HODGKISON et al., 2007; RIEGER; JAKOB, 1988; THIES; KALKO; SCHNITZLER, 1998). Uma série de estudos já foram realizados para analisar esta grande acuidade, com análises morfológicas (e.g. BHATNAGAR; KALLEN, 1975), testes em laboratório (e.g. LASKA, 1990a, 1990b), cativeiro (HODGKISON et al., 2013; PAROLIN; MIKICH; BIANCONI, 2015; TANG et al., 2007; THIES; KALKO; SCHNITZLER, 1998; ZHANG et al., 2014), experimentos e observações de campo (BIANCONI et al., 2007, 2012; MIKICH et al., 2003; RATCLIFFE, 1932; VAN DER PIJL, 1957), e até análises filogenéticas (HAYDEN et al., 2014), reforçando a importância do olfato nestes frugívoros.

Artibeus, *Carollia* e *Sturnira* e restauração florestal

Dentre os filostomídeos frugívoros, alguns gêneros se destacam por sua diversidade e abundância nos ecossistemas neotropicais e consequentemente pelo grande número de estudos sobre sua ecologia e biologia: *Artibeus* Leach, 1821, *Carollia* Gray, 1838 e *Sturnira* Gray, 1842 (e.g. Fleming 1988, Laska 1990, Simmons & Voss 1998, Thies et al. 1998, Kalko & Handley Jr 2001, Andrade et al. 2013, Saldaña-Vázquez 2014). A dieta destes filostomídeos conta com mais de 170 gêneros de frutos, pertencentes a 62 famílias botânicas (PAROLIN; BIANCONI; MIKICH, 2016). Além disso, uma das questões mais estudadas é a marcada preferência destes gêneros por determinados frutos: *Artibeus* spp. por *Ficus* L. (Moraceae), *Carollia* spp. por *Piper* L. (Piperaceae) e *Sturnira* spp. por *Solanum* L. (Solanaceae) (ANDRADE et al., 2013; FLEMING, 1988; GIANNINI, 1999; HANDLEY; WILSON; GARDNER, 1991; MORRISON, 1978; PAROLIN, 2013; PAROLIN; BIANCONI; MIKICH, 2016; SÁNCHEZ et al., 2012). A relação destes morcegos e frutos é consistente tanto no tempo, quanto no espaço, ou seja, ao longo de um ciclo anual e nas diferentes localidades onde os morcegos se distribuem (PAROLIN, 2013; PAROLIN; BIANCONI; MIKICH, 2016).

Os estudos de Mikich et al. (2003) e Bianconi et al. (2007), utilizaram estes conceitos quando testaram a atração de morcegos frugívoros com o uso de apenas óleos essenciais de frutos zoocóricos. Tanto em áreas florestais (MIKICH et al., 2003), como em áreas agrícolas abertas (BIANCONI et al., 2007), foram instaladas redes-de-neblina tratadas com frutos miméticos contendo óleos essenciais brutos de *Ficus* e *Piper* maduros, intercaladas com redes com frutos miméticos contendo apenas água destilada. O número de capturas, envolvendo principalmente os gêneros *Artibeus*, *Carollia e Sturnira*, foi significativamente maior nas redes contendo o odor dos frutos.

Com estes resultados positivos, os autores utilizaram estes três gêneros de morcegos como modelos na proposição de uma ferramenta para recuperação de áreas degradadas, que é baseada na atração destes animais para aumentar o banco de sementes em locais degradados (BIANCONI et al., 2007). Estudos ressaltaram ainda que estes animais naturalmente cruzam estas áreas na busca por recurso em fragmentos florestais (BIANCONI, 2009) e que na presença do estímulo odorífero, eles gastam um tempo buscando a fonte alimentar, aumentando assim a probabilidade de defecar sementes no seu entorno (BIANCONI et al., 2012). Por fim, experimentos em cativeiro ainda mostraram que estes gêneros de morcegos respondem significativamente mais a materiais inertes contendo apenas os óleos essenciais extraídos de seus frutos preferidos do que de frutos inteiros de outras espécies (PAROLIN, 2013; PAROLIN; MIKICH; BIANCONI, 2015).

Esta ferramenta de recuperação de áreas degradadas é particularmente importante no momento em que o planeta se encontra em relação às suas áreas naturais. O cenário não é animador: o desmatamento chegou a 129 milhões de hectares em todo o Planeta no ano de 2015 (FAO, 2015). Desta forma, a demanda por novas, de baio custo e eficientes técnicas de recuperação de áreas degradadas, principalmente aquelas com potencial de recuperar não apenas a função, mas também a forma dos ecossistemas originais, também aumenta. No entanto, a maioria das técnicas de restauração utilizadas não inclui a diversidade de plantas e formas de vida normalmente consumidas e dispersas por animais (CHAZDON, 2008).

O amplo uso dessa técnica de recuperação de áreas degradadas atualmente esbarra na falta de identificação dos compostos responsáveis pela atração dos filostomídeos frugívoros, permitindo a sua síntese. Para elucidar este e outros aspectos, a presente tese foi dividida em quatro capítulos. O Capítulo I trata dos óleos essenciais presentes em frutos preferidos, consumidos e não consumidos pelos morcegos filostomídeos dos gêneros *Artibeus*, *Carollia* e *Sturnira* presentes no município de Fênix, Paraná, Brasil. A composição deste óleos foi comparada de modo a analisar padrões de escolha olfativa, como compostos-chave para a identificação dos frutos preferidos, ou mesmo compostos comuns entre os frutos mais consumidos.

Nesta linha, o Capítulo II investiga os compostos com maior importância na atratividade, utilizando como exemplo a interação de *Carollia perspicillata* (Linnaeus, 1758) e *Piper gaudichaudianum* Kunth. Utilizando a análise cromatográfica na forma de uma técnica que denominamos “envelhecimento do óleo” (*oil aging*), combinada com experimentos em cativeiro de dupla escolha, analisamos o papel de diferentes VOCs na atração destes morcegos. A partir dos resultados, pudemos trazer informações inéditas sobre a interação dos óleos essenciais com morcegos frugívoros, além de incrementar e direcionar a ferramenta de restauração.

O Capítulo III contém uma compilação e análise da dieta frugívora de Pteropodidae, morcegos do Velho Mundo, em toda sua distribuição nas diferentes regiões zoogeográficas. Com isto, buscou-se um panorama geral sobre o consumo frugívoro dos gêneros de Pteropodidae, analisando sua dieta ao longo de sua distribuição e se há consistência desta dieta em relação aos reinos zoogeográficos do Velho Mundo.

Por fim, no Capítulo IV buscamos gêneros de Pteropodidae com potencial de atuar como vetores da técnica de restauração na África, Oceania ou Ásia, tal como o fazem *Artibeus*, *Carollia* e *Sturnira* nas Américas. Seguindo a proposta do filostomídeos, o primeiro passo foi selecionar os gêneros de raposas-voadoras que apresentavam ampla dieta frugívora, conferindo-lhes o potencial de dispersar vários grupos de plantas e espécies, mas também com uma marcada preferência por um gênero ou espécie de fruto, para a utilização do seu óleo essencial como atrativo. Para isto foram selecionados a partir dos dados do Capítulo III, os gêneros de morcego que atendessem aos requisitos citados, apresentando e discutindo suas características.

O conjunto destas informações fornece subsídios claros para implantação da técnica de recuperação de ecossistemas florestais degradados na Região Neotropical, além da sugestão de sua expansão para as demais regiões tropicais do Mundo.

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CAPÍTULO 1 - ÓLEOS ESSENCIAIS DE FRUTOS CONSUMIDOS POR MORCEGOS: EXISTE ALGUM PADRÃO?

RESUMO

Os compostos orgânicos voláteis (VOCs) são importantes na comunicação mutualística entre plantas e dispersores, particularmente em se tratando de morcegos frugívoros, uma vez que estes animais utilizam principalmente o olfato na seleção dos frutos. Apesar disso, pouco se sabe sobre a escolha olfativa destes animais, como quais são os compostos envolvidos na atração. Desta forma, este estudo buscou responder às seguintes questões: i. Os frutos mais consumidos por um gênero de morcego apresentam compostos comuns nos seus óleos essenciais?; e, ii. Os óleos essenciais dos frutos preferenciais apresentam particularidades ou compostos-chave que os identificam para os morcegos frugívoros? Para tanto, analisamos a composição dos óleos essenciais de 18 espécies de frutos classificados em preferenciais, consumidos e não consumidos pelos três principais gêneros de filostomídeos frugívoros *Artibeus*, *Carollia* e *Sturnira*. Os resultados mostraram que os frutos preferidos pelos morcegos apresentam similaridades na composição dos seus VOCs e que alguns compostos se destacam entre esses frutos, como Óxido de cariofileno, Juniperol, Guaia-3,9-dien-11-ol <cis->, Longifolol e Khusimol. Estas informações trazem avanços significativos à ecologia química e à elucidação dos mecanismos de comunicação entre animais e plantas e de atração de morcegos dispersores aos frutos.

Palavras-chave: Chiroptera, compostos orgânicos voláteis, frugivoria, olfato, terpenóides.

INTRODUÇÃO

A interação mutualística entre animais e plantas traz benefícios nutritivos para os animais e maior eficiência na polinização e na dispersão dos propágulos (WHEELWRIGHT; ORIANS, 1982). As plantas possuem artifícios visuais, morfológicos e olfativos, como a emissão de compostos voláteis, os chamados óleos essenciais, que atraem os potenciais dispersores (DUDAREVA et al., 2006). Estes compostos orgânicos voláteis (VOCs) são considerados aleloquímicos sinomônios, pois são compostos envolvidos na comunicação interespecífica e trazem benefícios tanto para o emissor, quanto para o receptor (HICK; LUSZNIAK; PICKETT, 1999; WHITTAKER; FEENY, 1971).

Morcegos frugívoros possuem o olfato como o principal sentido responsável pela localização e seleção de seus recursos alimentares (THIES; KALKO; SCHNITZLER, 1998). Essa característica levou um grupo de pesquisadores a testar a possibilidade de atração de filostomídeos frugívoros em ambientes florestais (MIKICH et al., 2003), áreas agrícolas e abertas, utilizando unicamente óleos essenciais extraídos de frutos presentes em sua dieta. Os resultados positivos desses experimentos levaram à proposição de uma ferramenta para restauração de áreas degradadas, constituída na atração destes vetores de dispersão com o uso de óleos essenciais, aumentando assim a chuva de sementes (BIANCONI et al., 2007, 2012). Os morcegos dos gêneros *Artibeus*, *Carollia* e *Sturnira* foram usados como base para o delineamento da ferramenta, uma vez que são frugívoros bem estudados, abundantes e amplamente distribuídos na região Neotropical, além de possuírem vasta base de dados sobre sua acuidade olfativa e preferência alimentar, esta relacionada aos gêneros *Ficus*, *Piper* e *Solanum*, respectivamente (ANDRADE et al., 2013; FLEMING, 1988; LASKA, 1990; PAROLIN; BIANCONI; MIKICH, 2016; PAROLIN; MIKICH; BIANCONI, 2015; RIEGER; JAKOB, 1988; SÁNCHEZ; GIANNINI; BARQUEZ, 2012; THIES; KALKO; SCHNITZLER, 1998).

Apesar da eficiência dos experimentos acima, poucos são os estudos que abordam a comunicação entre animais e plantas por meio dos VOCs. A maioria dos estudos está relacionada à emissão de compostos induzidos pela herbivoria facilitando a comunicação entre plantas ou para atração de polinizadores (e.g. DUDAREVA et al., 2006; FARRÉ-ARMENGOL et al., 2013; SIMPRAGA; TAKABAYASHI; HOLOPAINEN, 2016); ademais, quanto aos frutos, escassas são as informações sobre a sua interação com os dispersores e ainda poucos são os estudos sobre a composição de óleos essenciais de espécies não-cultivadas importantes na dieta de animais silvestres (BORGES, 2015; DUDAREVA et al., 2013).

Os estudos que tratam dos VOCs e quirópteros, foco deste trabalho, envolvem a atração *per se*, as preferências olfativas quanto às espécies de frutos e até em alguns casos, os compostos presentes nestes óleos essenciais (BIANCONI et al., 2007; BORGES; BESSIÈRE, 2008; HODGKISON et al., 2007, 2013; LUFT; CURIO; TACUD, 2003; MIKICH et al., 2003; PAROLIN; MIKICH; BIANCONI, 2015).

Entretanto, as características da atração e os compostos envolvidos são pouco explorados (e.g. HODGKISON et al., 2013), dificultando a identificação de um possível padrão em relação aos compostos mais ou menos importantes para a interação entre VOCs e morcegos. Assim, este estudo buscou analisar a composição dos óleos essenciais de 12 espécies de frutos que os morcegos frugívoros dos gêneros *Artibeus*, *Carollia* e *Sturnira* consomem em maior ou menor quantidade, ou até mesmo não consomem. Estas informações permitiram testar as seguintes hipóteses: i. os frutos mais consumidos por um determinado gênero de morcego apresentam compostos comuns nos seus óleos essenciais; e, ii. óleos essenciais dos frutos preferenciais apresentam particularidades ou compostos-chave que os identificam para os morcegos frugívoros.

MÉTODOS E MATERIAIS

Área de estudo

Os frutos foram coletados em fragmentos de Floresta Atlântica no município de Fênix (23° 55’ S - 51° 57’ W), centro-oeste do Paraná, Brasil. O clima da região é considerado segundo a classificação de Köppen como subtropical úmido mesotérmico (*Cfa*), com temperaturas médias anuais variando de 16 a 29 °C e precipitação anual entre 1,400 e 1,500 mm (MAACK, 2012). Esta região foi palco de vários estudos envolvendo a relação morcego-planta, como sobre a composição e fenologia das espécies zoocóricas (MIKICH; SILVA, 2001), além daqueles que testaram a atração de morcegos frugívoros por óleos essenciais (BIANCONI et al., 2007, 2012; MIKICH et al., 2003).

Frutos

Os doze frutos zoocóricos utilizados para análise dos seus óleos essenciais foram selecionados com base na sua oferta na área de estudo e na dieta dos morcegos dos gêneros *Artibeus*, *Carollia* e *Sturnira* na região de Fênix, entre os anos de 2000 e 2008 (BIANCONI; MIKICH in litt.). Estes dados se referem a sementes identificadas a partir da coleta de fezes de morcegos capturados em redes-de-neblina, identificados e deixados em sacos de pano individuais por, pelo menos, uma hora para defecarem. Os registros de consumo correspondem à presença da espécie em uma amostra fecal de morcego, independente do número de sementes. *Artibeus fimbriatus* Gray, 1838, *A. planirostris* (Spix, 1823), *A. lituratus* (Olfers, 1818), *Carollia perspicillata* (Linnaeus, 1758) e *Sturnira lilium* (E. Geoffroy, 1810) foram as espécies utilizadas neste estudo, mas apenas os gêneros foram tratados separadamente. Como o número de registros de consumo é muito variável, foi utilizada uma escala de consumo, que variou 0 a 10, para padronizar esses valores. Às espécies de frutos sem registro de consumo foi atribuída a classe "0" e à espécie de fruto com maior número de registros/gênero de morcego foi atribuída a classe "10", sendo as classes intermediárias obtidas por regra de três simples (Tabela 1).

Tabela 1. Número de registros (reg) e classe de consumo das espécies de frutos investigados em relação à dieta dos gêneros de morcegos *Artibeus*, *Carollia* e *Sturnira* no município de Fênix entre os anos de 2000 e 2008 com base em Bianconi e Mikich in litt..

Frutos	Sigla	*Artibeus*		*Carollia*		*Sturnira*	
		Reg	Classe	Reg	Classe	Reg	Classe
Campomanesia xanthocarpa O.Berg	CX	0	0	0	0	0	0
Cecropia glaziovii Snethl.	CG	45	1	9	1	0	0
Euterpe edulis Mart.	EE	0	0	0	0	0	0
Ficus glabra Vell.	FG	195	5	9	1	5	4
Ficus insipida **Willd**.	FI	451	**10**	17	2	6	5
Morus nigra L.	MN	0	0	1	1	0	0
Piper gaudichaudianum **Kunth**	PG	42	1	146	**10**	6	5
Piper hispidum Sw.	PH	19	1	87	6	4	4
Psidium guajava L.	PS	1	1	0	0	0	0
Psychotria carthagenensis Jacq.	PC	0	0	0	0	0	0
Solanum caavurana **Vell.**	SC	61	1	20	2	13	**10**
Solanum granulosoleprosum Dunal	SG	10	1	3	1	8	7

Extração e análise dos óleos essenciais

Óleos essenciais foram extraídos por meio da técnica de hidrodestilação, que se baseia no aquecimento do material vegetal (frutos) em água, fazendo com que os produtos voláteis sejam arrastados junto com o vapor por um sistema tipo Clevenger, conforme descrito em Bianconi et al. (2008). A fervura foi estabelecida em quatro horas, com 200 g de fruto, sendo o óleo obtido recuperado em éter etílico.

Até sua leitura no cromatógrafo, os óleos foram mantidos em ampolas de vidro vedadas e congeladas (-15°C).

A comparação dos óleos essenciais foi realizada em triplicata, usando cromatografia gasosa. As amostras foram introduzidas via injetor split/splitless (10 a 100 µL, 240 °C), sendo dissolvidas em 0,5 mL de acetato de etila e analisadas usando um aparelho Focus CG de cromatografia gasosa acoplado a um Polaris Q íon trap (Thermo), equipados com uma coluna capilar Thermo DBS MS (60 m x 0.25 mm, 0.25 µm). O forno do GC foi programado de 60 até 250 ºC min^{-1} a 3 °C min-1, e mantido a 250 °C durante 5 min. Hélio, a um fluxo constante de 1,0 mL min-1, foi o gás carregador. A temperatura do injetor e da linha de transferência foi de 240 °C e 250 °C, respectivamente. O Polaris Q íon trap foi operado no modo positivo a 70 eV de varredura da faixa de *m / z* 50-650 em um tempo de escaneamento total de 0,58 de emissão de 250 mA de corrente.

Os cromatogramas gerados foram comparados de acordo com a área formada pelos seus picos, excluindo-se aqueles representados por menos de 0.1% na amostra total, que não fossem de óleo essencial ou com sinal-ruído abaixo de 50 (i.e. valor reproduzido entre a amostra sob análise e uma amostra branca para detectar falhas na leitura). Para a identificação dos componentes foi empregado o cálculo dos índices de Kovats (IK), utilizando como base um cromatograma resultante da injeção de uma série homóloga de n-alcanos (C_7 a C_{30}). O IK calculado para cada composto foi comparado com valores da literatura (ADAMS, 2007).

Para comparar os óleos essenciais, os valores de área dos picos encontrados foram normalizados e transformados ($-\log_{10}$). A correlação entre a escala de consumo das espécies de frutos pelos gêneros de morcegos e as variáveis (compostos) foi realizada por meio de uma *Partial Least Squares* (PLS) no software Unscrambler X 10.4.1. Esta análise é similar à *Principle Component Analysis*, mas utiliza uma nova variável ou fator para correlacionar os dados, de modo a explicar ao máximo a sua variação (DORMANN et al., 2013). Por fim, utilizamos o Two-Way ANOVA para comparar os compostos importantes resultantes da PLS para cada gênero de morcego entre as espécies de frutos.

RESULTADOS

Ao todo foram detectados 145 compostos nos óleos essenciais das 12 espécies de frutos. *Campomanesia xanthocarpa* apresentou o maior número de compostos (n = 77), seguido de *F. glabra* (n = 41) e *P. gaudichaudianum* (n = 41), *C. glaziovii* (n = 37), *F. insipida* (n = 36), *P. hispidum* (n = 35), *S. caavurana* (n = 34), *P. carthagenensis* (n = 26), *M. nigra* (n = 17), *P. guajava* (n =1 2), *E. edulis* (n = 7) e *S. granulosoleprosum* (n = 6).

Cada análise de PLS apresentou uma conformação diferente, de acordo com a escala de consumo de cada morcego. As Figuras 1, 2 e 3 apresentam as correlações obtidas, os gráficos dos scores do Factor 1 e correlation loadings para *Artibeus*, *Carollia* e *Sturnira*, respectivamente. Destacam-se as retas de correlação indicando que entre consumo e composição dos óleos existe uma tendência linear (Figuras 1A, 2A e 3A). É possível observar que em cada um dos casos há a separação dos frutos mais consumidos dos demais em valores positivos de Factor 1.

Para *Artibeus*, há destaque para os frutos de *F. insipida* e *F. glabra*, espécies mais consumidas por este gênero (Tabela 1, Figura 1B), além de certa correlação com *P. gaudichaudianum* e *S. caavurana*. Para *Carollia* (Figura 2B), há a separação dos óleos essenciais dos frutos mais consumidos (*P. gaudichaudianum* e *P. hispidum*), além de alguma relação com *C. xanthocarpa*, *F. insipida* e *S. caavurana.* No caso de *Sturnira*, observa-se o destaque para *S. caavurana*, *P. gaudichaudianum*, *P. hispidum* e *F. insipida*, sem diferenciação aparente para a primeira espécie, a mais consumida por esse morcego. Além disso, a espécie *S. granulosoleprosum,* um fruto frequentemente consumido por *Sturnira* (Tabela 1, Classe 7), aparece como um possível "*outlier*".

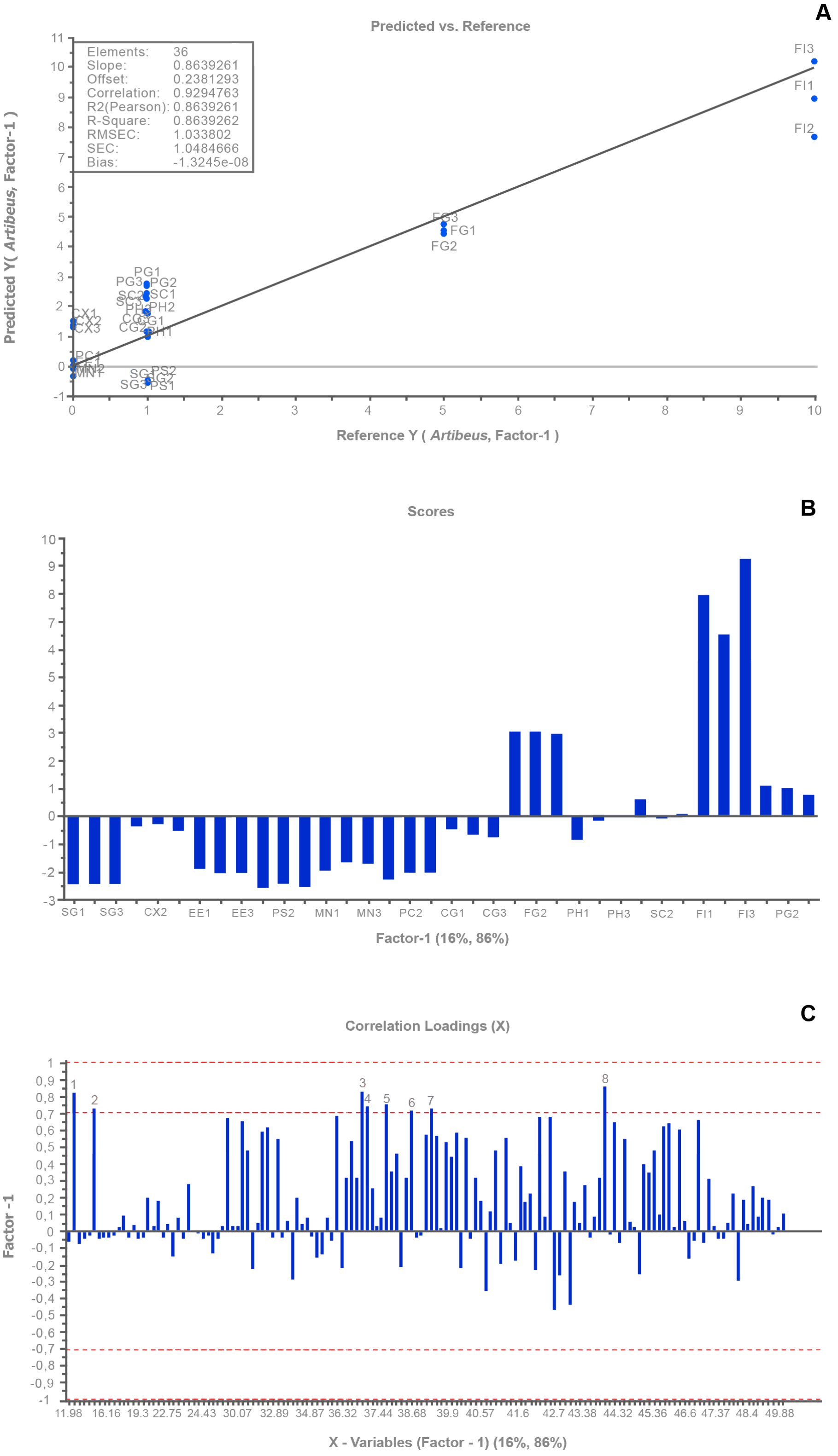

Figura 1. Análise PLS dos GC-MS cromatogramas dos óleos essenciais das 12 espécies de frutos. Reta de calibração (A), Scores do Factor 1 (B) e o correlation loadings (C) para *Artibeus*. Siglas das espécies encontram-se na Tabela 1 e dos compostos na Tabela 2.

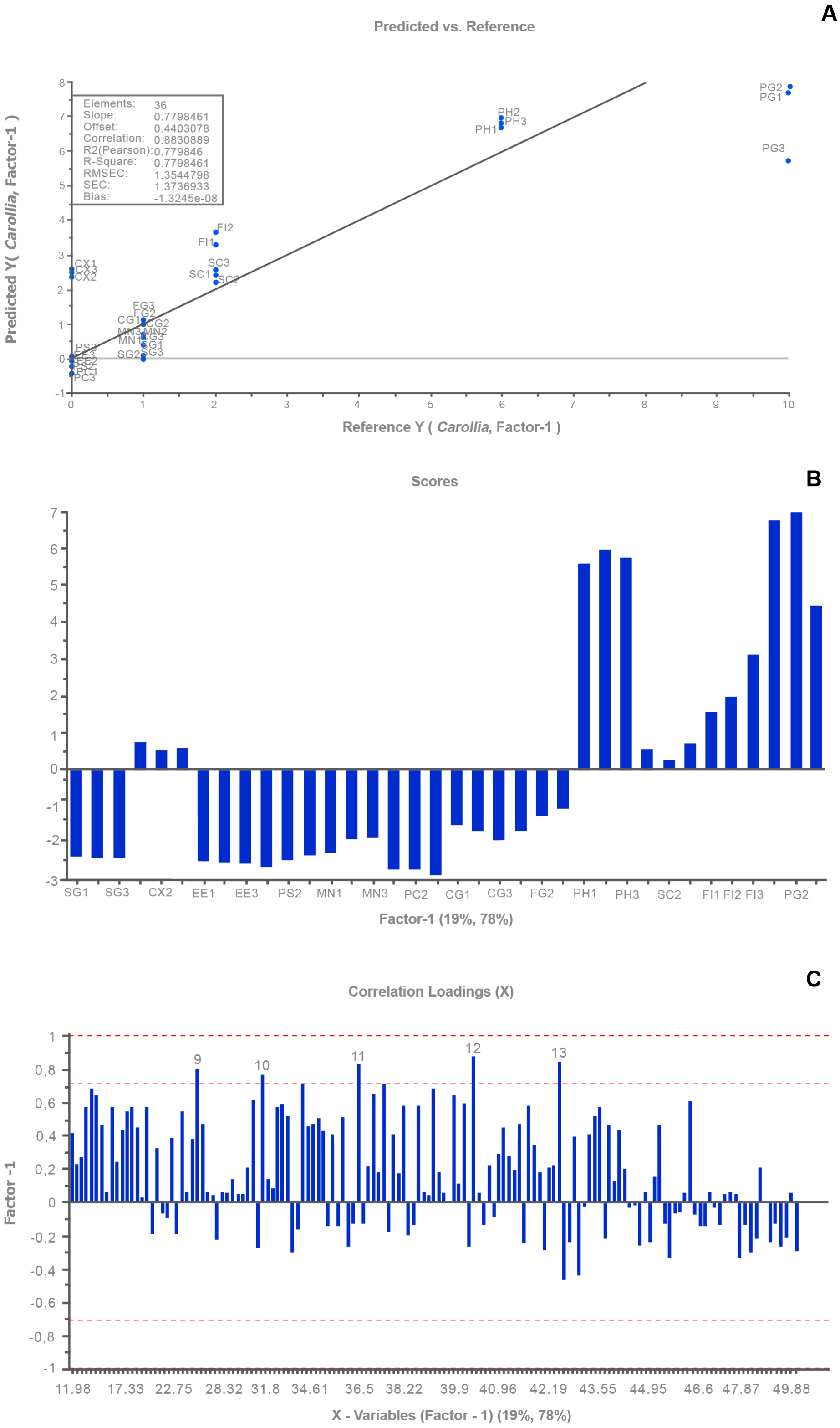

Figura 2. Análise PLS dos GC-MS cromatogramas dos óleos essenciais das 12 espécies de frutos. Reta de calibração (A), Scores do Factor 1 (B) e o correlation loadings (C) para *Carollia*. Siglas das espécies encontram-se na Tabela 1 e dos compostos na Tabela 2.

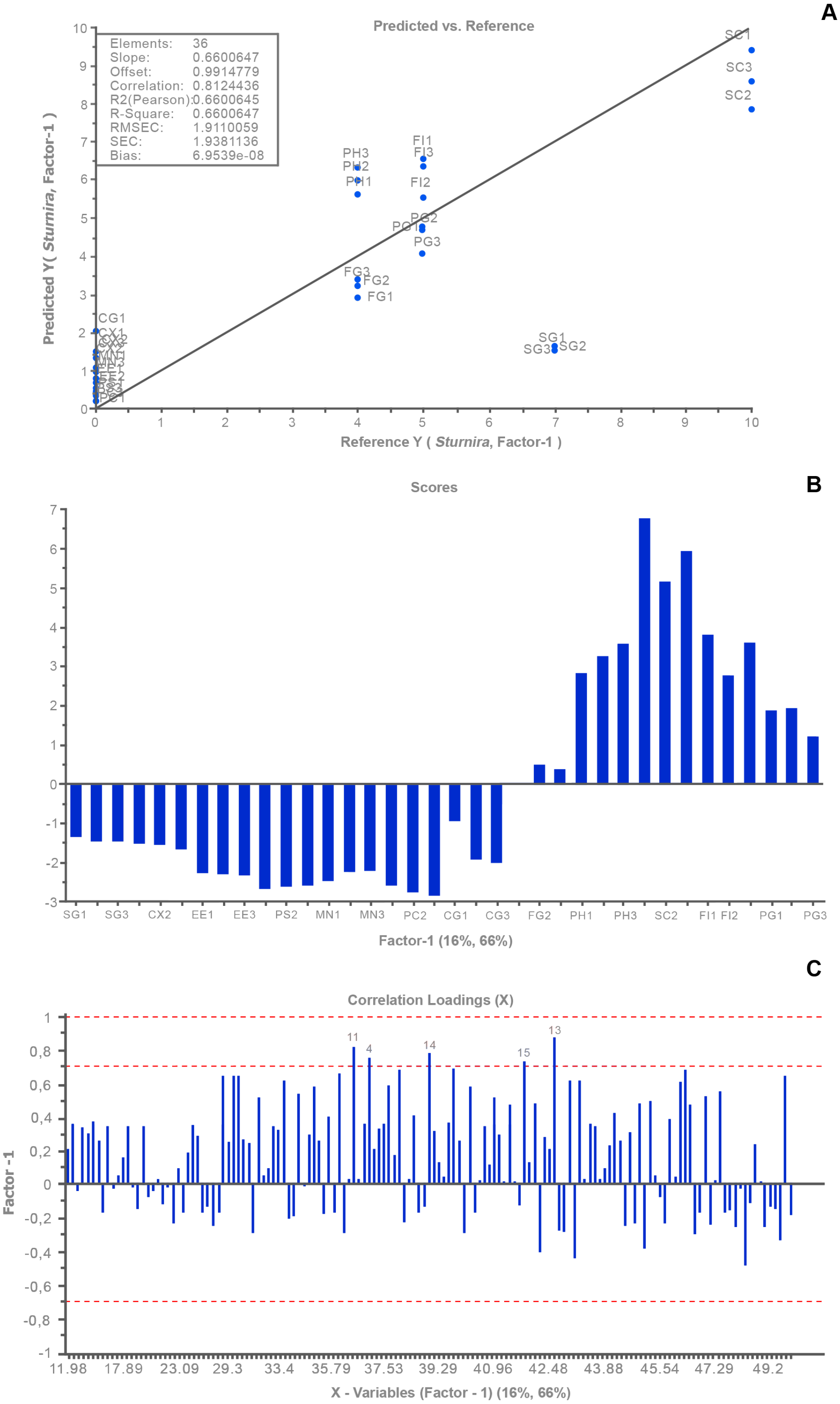

Figura 3. Análise PLS dos GC-MS cromatogramas dos óleos essenciais das 12 espécies de frutos. Reta de calibração (A), Scores do Factor 1 (B) e o correlation loadings (C) para *Sturnira*. Siglas das espécies encontram-se na Tabela 1 e dos compostos na Tabela 2.

Quando se analisa o biplot de correlation loadings, observa-se que alguns compostos foram mais importantes na formação e separação dos óleos essenciais das 12 espécies de frutos (Figuras 1C, 2C e 3C). A Tabela 2 apresenta estes compostos responsáveis pela correlação e com alta relação com os frutos preferenciais. Vale destacar que dois compostos importantes para *Artibeus* e *Carollia,* Juniperol e Óxido de cariofileno, também se mostraram importantes para *Sturnira*. O composto 3 está como "Não identificado" pois não foi possível encontrar seu espectro de massas na base de dados.

Tabela 2. Compostos importantes na separação dos frutos consumidos, número correspondente na Figura 1 (N^o), tempo de retenção nos cromatogramas (TR), Índice de Kovats (IK) e classificação química.

Composto	N^o	TR	IK^1	IK^2	Classe
Cresol <p->	1	13.38	1075	1076	Fenol
Ocimeno <allo->	2	15.98	1133	1132	Monoterpeno
Thujanol acetato <3->	9	23.73	1300	1295	NI
Gurjuneno <γ->	10	31.8	1479	1477	Sesquiterpeno
Óxido de cariofileno	11	36.5	1586	1583	Sesquiterpeno
Não identificado (NI)	3	36.82	1594	ND	NI
Juniperol	4	37.02	1599	1599	Sesquiterpeno
Cubenol <1,10-di-epi->	5	37.9	1621	1619	Sesquiterpeno
Acorenol <β->	6	38.68	1641	1637	Sesquiterpeno
Guaia-3,9-dien-11-ol <cis->	14	39.06	1650	1649	Sesquiterpeno
Exalatacin	7	39.22	1654	1656	Fenol
Elemol acetato	12	40.33	1682	1680	Sesquiterpeno
Longifolol	15	41.74	1717	1714	Sesquiterpeno
Khusimol	13	42.7	1743	1742	Sesquiterpeno
Amorpha-4,7(11)-dieno<2-a-hydroxy->	8	44.06	1778	1776	Sesquiterpeno

^{1}Índice de Kovats calculado. ^{2} Índice de Kovats encontrado (ADAMS, 2007).

O teste ANOVA mostrou que estes compostos importantes para cada morcego variam entre frutos com classes de consumo distintas. Para *Artibeus*, os compostos Cresol <p->, "Não identificado" e Exalatacin só estão presentes em *F. insipida*, o fruto mais consumido por este gênero de morcego na localidade estudada (Tabela 3). Observa-se que *F. insipida* apresenta maior área relativa do que as outras espécies de fruto que apresentam o mesmo composto, com algumas exceções em espécies de fruto e compostos em que a diferença não foi significativa: *C. glaziovii* (Cubenol <1,10-di-epi->), *F. glabra* (Ocimeno <allo->, Juniperol e Cubenol <1,10-di-epi->), *P. hispidum* (Ocimeno <allo-> e Juniperol), *P.*

gaudichaudianum (Cubenol <1,10-di-epi-> e Acorenol <β->) e *S. caavurana* (compostos Juniperol e Cubenol <1,10-di-epi->) (Tabela 3). Para *Carollia*, as espécies *P. hispidum* e *P. gaudichaudianum* se destacam, com a segunda sendo com a área significativamente maior que as outras espécies para o composto Thujanol acetato <3->; nos outros compostos só não é maior que *C. xanthocarpa* (Gurjuneno <γ-> e Óxido de cariofileno), *F. glabra* (Khusimol), *F. insipida* (compostos Elemol acetato e Khusimol) e *S. caavurana* (Óxido de cariofileno e Khusimol) (Tabela 4). Por fim, para *Sturnira* (Tabela 5), apesar de não ter nenhum composto único para *S. caavurana* (fruto mais consumido), o cenário é similar aos anteriores. Esta solanácea apresentou maior área para os cinco compostos apresentados em relação a várias espécies, retirando *C. xanthocarpa* (Óxido de cariofileno e Guaia-3,9-dien-11-ol <cis->), *C. glaziovii* (Juniperol e Guaia-3,9-dien-11-ol <cis->), *F. glabra* (Juniperol e Khusimol), *F. insipida* (Óxido de cariofileno, Guaia-3,9-dien-11-ol <cis->, Longifolol e Khusimol), P. *hispidum* (Guaia-3,9-dien-11-ol <cis-> e Longifolol), *P. gaudichaudianum* (Óxido de cariofileno, Juniperol, Longifolol e Khusimol), *P. guajava* (Juniperol) e *S. granulosoleprosum* (Longifolol).

Tabela 3. Média, desvio padrão da área relativa dos compostos importantes na PLS de *Artibeus* para as 12 espécies de frutos. As letras acompanham os valores significativamente similares por composto entre as diferentes espécies de frutos de acordo com a análise de variância (Two-Way ANOVA).

Compostos	Espécies de frutos											
	CG	CX	EE	FG	FI	MN	PG	PH	PC	PS	SC	SG
Cresol <p->	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	2.5 ± 0.4^{b}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Ocimeno <allo->	0.3 ± 0.2^{a}	1.6 ± 0.04^{b}	1.3 ± 0.2^{b}	1.8 ± 0.1^{bc}	2.6 ± 0.3^{cd}	0 ± 0^{e}	$1.7\pm0.6b^{cd}$	1.8 ± 0.2^{bc}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}
Não identificado	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	2.2 ± 0.7^{b}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Juniperol	0.8 ± 0.3^{abd}	0.2 ± 0.06^{a}	0 ± 0^{a}	1.4 ± 0.06^{bcd}	2.1 ± 0.3^{cd}	0 ± 0^{a}	1.2 ± 0.2^{d}	1.8 ± 0.06^{acd}	0 ± 0^{a}	0.9 ± 0.6^{abd}	1.3 ± 0.08^{cd}	0 ± 0^{a}
Cubenol <1,10-di-epi->	1.2 ± 0.1^{acd}	0.7 ± 0.05^{abd}	0 ± 0^{b}	2.1 ± 0.2^{cd}	2.0 ± 0.1^{cd}	0 ± 0^{b}	1.3 ± 0.1^{d}	0.7 ± 1.3^{bde}	0 ± 0^{b}	0 ± 0^{b}	1.3 ± 1.1^{cde}	0 ± 0^{b}
Acorenol <β->	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	2.2 ± 0.7^{b}	0 ± 0^{a}	2.0 ± 0.2^{b}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Exalatacin	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	1.5 ± 1.3^{b}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Amorpha-4,7(11)-dieno<2-a-hydroxy->	0.62 ± 0.53^{ab}	1.07 ± 0.15^{b}	0 ± 0^{a}	1.2 ± 0.01^{b}	2.5 ± 0.6^{c}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}

Tabela 4. Média, desvio padrão da área relativa dos compostos importantes na PLS de *Carollia* para as 12 espécies de frutos. As letras acompanham os valores significativamente similares por composto entre as diferentes espécies de frutos de acordo com a análise de variância (Two-Way ANOVA).

Compostos	Espécies de frutos											
	CG	CX	EE	FG	FI	MN	PG	PH	PC	PS	SC	SG
Thujanol acetato <3->	0±0^{a}	0.5±0.05^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	1.0±0.9^{b}	2.2±0.4^{c}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Gurjuneno <γ->	0±0^{a}	1.7±0.06^{bc}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	2.1±0.4^{b}	1.3±0.02^{c}	0±0^{a}	0±0^{a}	1.4±0.12^{c}	0±0^{a}
Óxido de cariofileno	0±0^{a}	1.6±0.03^{bd}	0±0^{a}	0±0^{a}	2.4±0.3^{ce}	0±0^{a}	1.7±0.2^{d}	2.5±0.3^{e}	0±0^{a}	0±0^{a}	2.0±0.1^{cd}	0±0^{a}
Elemol acetato	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	2.0±0.04^{bc}	0±0^{a}	1.7±0.04^{c}	2.3±0.05^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Khusimol	0±0^{a}	0.6±0.1^{b}	0±0^{a}	1.0±0.05^{bd}	1.7±0.2^{cd}	0±0^{a}	1.3±0.05^{d}	1.9±0.01^{c}	0±0^{a}	0±0^{a}	1.3±0.1^{cd}	0±0^{a}

Tabela 5. Média, desvio padrão da área relativa dos compostos importantes na PLS de *Sturnira* para as 12 espécies de frutos. As letras acompanham os valores significativamente similares por composto entre as diferentes espécies de frutos de acordo com a análise de variância (Two-Way ANOVA).

Compostos	Espécies de frutos											
	CG	CX	EE	FG	FI	MN	PG	PH	PC	PS	SC	SG
Óxido de cariofileno	0±0^{a}	1.6±0.03^{bd}	0±0^{a}	0±0^{a}	2.4±0.3^{ce}	0±0^{a}	1.7±0.2^{d}	2.6±0.33^{e}	0±0^{a}	0±0^{a}	2.0±0.1^{cd}	0±0^{a}
Juniperol	0.9±0.31^{abd}	0.2±0.06^{a}	0±0^{a}	1.4±0.06^{bcd}	2.1±0.3^{cd}	0±0^{a}	1.2±0.2^{d}	1.8±0.06^{acd}	0±0^{a}	0.9±0.6^{abd}	1.3±0.08^{cd}	0±0^{a}
Guaia-3,9-dien-11-ol <cis->	1.3±0.04^{a}	1.3±0.08^{a}	0±0^{b}	1.2±0.02^{a}	1.4±0.2^{a}	0±0^{b}	1.3±0.3^{a}	1.7±0.08^{a}	0±0^{b}	0±0^{b}	1.8±0.1^{a}	0±0^{b}
Longifolol	1.1±0.13^{ac}	0.8±0.04^{a}	0±0^{b}	1.0±0.21^{ac}	1.5±0.2^{c}	0.8±0.7^{a}	1.4±0.2^{ac}	1.9±0.1^{c}	0±0^{b}	0±0^{b}	1.8±0.06^{c}	1.8±0.1^{c}
Khusimol	0±0^{a}	0.6±0.14^{b}	0±0^{a}	1.0±0.05^{bd}	1.7±0.2^{cd}	0±0^{a}	1.3±0.05^{d}	1.9±0.01^{c}	0±0^{a}	0±0^{a}	1.3±0.1^{cd}	0±0^{a}

Por fim, quando se observam apenas as espécies tratadas como preferidas, 18 são os compostos comuns entre elas, sendo em sua grande maioria sesquiterpenos (Tabela 6). Alguns destes compostos também estavam em destaque na PLS, como Óxido de cariofileno, Juniperol, Guaia-3,9-dien-11-ol <cis->, Longifolol e Khusimol.

Tabela 6. Compostos comuns para *Ficus insipida*, *Piper gaudichaudianum* e *Solanum caavurana*, seu tempo de retenção nos cromatogramas (TR), Índice de Kovats (IK) e classificação química.

Composto	TR	IK[1]	IK[2]	Classe
Muuroleno < γ ->	32.08	1484	1479	sesquiterpeno
Spathulenol	36.26	1581	1578	sesquiterpeno
Óxido de cariofileno	36.5	1586	1583	sesquiterpeno
Juniperol	37.02	1599	1599	sesquiterpeno
Khusimono	37.27	1605	1604	cetona
Cubenol <1,10-di-epi->	37.9	1621	1619	sesquiterpeno
Eudesmol <10-epi- γ ->	38.06	1625	1623	sesquiterpeno
Muurola-4,10(14)-dien-1-B-ol	38.22	1630	1631	sesquiterpeno
Guaia-3,9-dien-11-ol <cis->	39.06	1650	1649	sesquiterpeno
Juniperol acetato	40.58	1687	1685	sesquiterpeno
Botrydiol	40.85	1694	1690	sesquiterpeno
Amorpha-4,9-dien-14-al	41.31	1705	1704	sesquiterpeno
Longifolol	41.74	1717	1714	sesquiterpeno
Curcumenol	42.48	1737	1734	sesquiterpeno
Khusimol	42.7	1743	1742	sesquiterpeno
Amorpha-4,9-dieno <7,14-anhydro>	43.03	1752	1756	sesquiterpeno
Guaiazuleno	44.22	1782	1780	sesquiterpeno

[1]Índice de Kovats calculado. [2] Índice de Kovats encontrado (ADAMS, 2007).

DISCUSSÃO

Os dados deste estudo trazem importantes contribuições ao estudo das interações animal-planta e à ecologia química, além de confirmam as hipóteses de que (i) existem semelhanças entre óleos essenciais de frutos preferidos pelos filostomídeos frugívoros *Artibeus*, *Carollia* e *Sturnira* quando comparados aos óleos encontrados em outros frutos consumidos ou não por esses gêneros de morcegos e (ii) que existem compostos-chave que parecem caracterizar os frutos preferidos por estes morcegos. Estas resultados trazem avanços na elucidação dos mecanismos de comunicação entre animais e plantas e de atração dos frutos em relação a morcegos dispersores.

Os óleos essenciais – os VOCs - compreendem compostos produzidos em diferentes partes de uma planta, servindo como a sua comunicação com o meio (DUDAREVA et al., 2006; LI; FABIANO-TIXIER; CHEMAT, 2014). A sua composição é basicamente uma mistura de terpenóides, mas ainda ocorrem cetonas, álcoois, fenóis e aldeídos (ADAMS, 2007). De acordo com Gershenzon e Dudareva (2007), esta mistura de compostos secundários serve para aumentar a eficiência e especificidade da atração pelo emissor; a produção de apenas um ou dois compostos não teria o mesmo alcance. Isto corrobora a quantidade de compostos encontrados (N = 145) e as particularidades de cada espécie, sendo esta mistura de odores responsável pelo sabor de cada fruto, além de alertar e fornecer informações aos seus dispersores (DUDAREVA et al., 2006).

Aqui utilizamos a escala de consumo dos morcegos para explicar a variação na composição dos óleos essenciais de diferentes espécies de frutos. Assim sendo, os compostos importantes são aqueles que melhor explicam o maior consumo dos três morcegos. Quando se observam estes compostos, pode-se sugerir que eles estejam envolvidos na diferenciação dos frutos preferidos pelos morcegos, com a possibilidade de serem os compostos-chave para a sua precisa identificação. Para *Sturnira* foi possível observar que alguns compostos se repetiram em relação a *Artibeus* e *Carollia* - Juniperol e Óxido de cariofileno - além da menor separação dos frutos consumidos nos scores da PLS. Como já ressaltado, o gênero *Artibeus* possui maior consumo e registros na literatura para o gênero *Ficus*, *Carollia* para *Piper* e *Sturnira* para *Solanum* (e.g PAROLIN; BIANCONI; MIKICH, 2016). Experimentos em cativeiro também comprovaram a maior atração destes morcegos pelos óleos essenciais brutos destes frutos (PAROLIN, 2013; PAROLIN; MIKICH; BIANCONI, 2015), mas alguns experimentos envolvendo a espécie *S. lilium* não mostraram diferença significativa quando da oferta simultânea de óleo essencial de *P. gaudichaudianum* e *S. caavurana* (PAROLIN, 2013), reforçando este perfil intermediário se comparado com os dois outros gêneros de morcegos.

As comparações entre os frutos preferenciais mostraram 18 compostos comuns entre *F. insipida*, *P. gaudichaudianum* e *S. caavurana*. Borges e Bessière (2008) encontraram semelhanças entre a composição de VOCs de figos dispersos por mamíferos, sendo diferentes daqueles predominantemente dispersos por aves. O olfato em mamíferos e sua importância na obtenção de alimento é historicamente reconhecida (STODDART, 1980; VAN DER PIJL, 1957, 1982), em contrapartida, aves se destacam por sua visão aguçada para selecionar frutos maduros (STEIGER et al., 2008; VAN DER PIJL, 1982). Estas diferenças entre os vetores de dispersão podem explicar as semelhanças entre voláteis e os frutos mais consumidos pelos filostomídeos frugívoros, como uma possível forma de selecionar os seus dispersores.

Além dos frutos preferenciais pertencentes aos gêneros *Ficus*, *Piper* e *Solanum*, a espécie *C. xanthocarpa* também mostrou relação com os morcegos, principalmente *Carollia*, apesar de não ter registro de consumo para estes morcegos na área de Fênix e também na literatura (PAROLIN; BIANCONI; MIKICH, 2016). Entretanto, deve-se destacar que uma das formas mais comuns de análise de dieta de morcegos é pela identificação das sementes encontradas nas fezes (VOIGT et al., 2009); e a semente de *C. xanthocarpa* é considerada de tamanho grande (7.0 mm de comprimento por 6.0 mm de largura, a semente da morácea *F. glabra*, por exemplo, possui 1.2 mm de comprimento e 0.8 mm de largura - MIKICH; SILVA, 2001), sendo dificilmente engolida por estes filostomídeos. Ademais, em experimento com indivíduos de *A. lituratus* cativos, não houve diferença significativa no número de investidas aos estímulos quando ofertados com óleo essencial de *F. insipida* (fruto do gênero preferencial) e de *C. xanthocarpa* (PAROLIN, 2013), mostrando que a composição química do seu óleo essencial é também atraente a estes animais.

As demais espécies de frutos analisadas não tiveram correlação positiva nas análises. Com a exceção de *C. glaziovii* e *S. granulosoleprosum,* as outras espécies possuem pouco ou nenhum registro de consumo pelos animais em questão (BIANCONI; MIKICH in litt.; PAROLIN; BIANCONI; MIKICH, 2016), como o caso de *E. edulis*, considerada espécie-chave em florestas tropicais (MIKICH, 2002; TERBORGH, 1986 mas ver GALETTI; ALEIXO, 1998; PERES, 2000). *Solanum granulosoleprosum* é consumida por *Sturnira* e apesar de ser do mesmo gênero de *S. caavurana*, não ficou próxima desta nas análises. Isto pode se relacionar com a baixa quantidade de compostos encontrada (N = 6). Já as espécies cultivadas *M. nigra* e *P. guajava* muito importantes na dieta de diversas espécies de vertebrados nativos (LIM, 2012; MIKICH, 2002; MIKICH; SILVA, 2001), mas pouco estão presentes na dieta de filostomídeos frugívoros, podendo isto explicar a baixa relação entre os seus compostos voláteis com os demais frutos consumidos.

As classes dos compostos em destaque nos resultados pertencem aos fenóis, cetonas, monoterpenos e sesquiterpenos, com destaque para o último, com particularidades para cada análise. De acordo com Hodgkison et al. (2013), morcegos da espécie *Artibeus jamaicensis* Leach, 1821 possuem uma relação positiva com frutos dominados por monoterpenos, quando testados frente a óleos essenciais brutos de cinco diferentes espécies de *Ficus*. De acordo com Parolin et al. (in prep.), isolaram e testaram alguns compostos que se mostraram importantes no reconhecimento do fruto de *P. gaudichaudianum* por *C. perspicillata,* com destaque para o sesquiterpeno α-copaeno. Os autores ainda destacaram a diferença na função de monoterpenos e sesquiterpenos na atração, com os primeiros servindo como um sinal inicial e curto sobre a presença do recurso; e os sesquiterpenos, como um sinal mais duradouro e indicador de viabilidade do fruto.

A ideia de que os óleos essenciais são os responsáveis pela atração dos filostomídeos frugívoros até o seu recurso alimentar já foi explorada (BIANCONI et al., 2007, 2012; MIKICH et al., 2003; PAROLIN; MIKICH; BIANCONI, 2015), com a sugestão de que alguns compostos isolados destes óleos podem ser extraídos e ainda atrair estes morcegos (BIANCONI et al., 2007, 2012); e que os óleos essenciais de frutos preferidos e consumidos por estes animais poderiam ter compostos comuns (PAROLIN; MIKICH; BIANCONI, 2015), facilitando a atração. As informações aqui analisadas puderam comprovar estas ideias, além de mostrar que alguns compostos podem ser mais importantes na diferenciação da espécie de fruto pelo animal.

A base da ferramenta de restauração que busca atrair morcegos frugívoros para aumentar o banco de sementes de áreas degradadas é a utilização de óleos essenciais provenientes de frutos consumidos por morcegos como chamariz (BIANCONI et al., 2007, 2012). Para utilização em larga escala é importante que estas substâncias possam ser sintetizadas, então um primeiro passo seria conhecer estes compostos relacionados a atração. Testes em cativeiro, campo e laboratório com os compostos que aqui diferenciaram as espécies de frutos (e.g. Cresol <p-> para *F. insipida*), além dos compostos comuns (e.g. Muuroleno <γ-> e Óxido de cariofileno), podem trazer respostas quanto à relação desses gêneros de morcegos com os VOCs dos frutos que usam como recurso, além de incrementar a ferramenta supracitada.

Estudos como este que tratam da composição dos óleos essenciais de espécies zoocóricas, trazem novas informações ao escasso conhecimento sobre a interação química entre fruto e dispersor, acrescentando à ecologia química e interação animal-planta, além fornecer subsídios práticos para a técnica de restauração de áreas degradadas. Novos estudos devem ser realizados de modo entender a função dos compostos aqui destacados, além de testá-los frente aos morcegos frugívoros, analisando sua percepção, especificidade da identificação e resposta ao emissor.

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CAPÍTULO 2 - A MOLECULAR VIEW OF BAT-PLANT INTERACTIONS

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ABSTRACT

Fruit-eating bats are one of the most important seed dispersers in tropical forests. Even though most bats rely heavily on echolocation to navigate and forage, olfaction seems to be the main sense used by these bats to locate and select food in the dark of the nighttime forest. Indeed, previous studies have demonstrated that fruit bats may be able to discriminate, identify, and select volatile organic compounds and, significantly, might be able to track essential oils of their preferred fruits. However, the specific role played by different compounds in attracting these seed-dispersers is largely unknown. Here, we use chromatographic analysis and coupled attraction trails to investigate the molecular basis of fruit foraging in two neotropical fruit bats. These bats are able to discriminate two classes of compounds, monoterpenes and sesquiterpenes, which play specific roles in leading bats to mature fruits. Monoterpenes traveling within odor plumes provide the initial signal, allowing bats to identify fruiting trees while flying around the forest. The next molecular signal is provided by sesquiterpenes: heavier, less volatile and longer-lived compounds that are present until fruit senescence and which provide information on fruit ripeness. These findings provide a unique molecular perspective to bat-plant communication with important implications for tropical forest restoration. Specifically, simple mixtures of commercially available terpenes could replace expensive wild fruit extracts as a means to attract seed-dispersing bats into degraded landscapes.

Key words: Animal-plant interaction, Chiroptera, essential oils, olfaction, terpenes

INTRODUCTION

Fruit bats are known to locate and select mature fruit using primarily olfactory signals (LASKA, 1990a, 1990b), but mechanisms underlying this attraction are largely unknown. Here we examine bat-fruit interactions at a molecular level to understand how they identify and choose fruits while foraging in low light conditions.

Compared to insectivorous bats, fruit-eating bats have larger olfactory bulbs and a greater olfactory receptor surface area (BHATNAGAR, 1975; BHATNAGAR; KALLEN, 1975; JONES; BININDA-EMONDS; GITTLEMAN, 2005). Experiments with wild and captive New World fruit bats (BIANCONI et al., 2007, 2012; MIKICH et al., 2003; PAROLIN; MIKICH; BIANCONI, 2015) show that they can be attracted using only essential oils - concentrated hydrophobic liquids containing a volatile aroma (KNUDSEN et al., 2006; VAN DER PIJL, 1957) - extracted from mature chiropterochoric fruit. In plants, these volatile organic compounds (VOCs) play a major role in communication with mutualistic animals (BALDWIN et al., 2006), signaling both the stage of fruit maturation and the nutritional resources on offer (RODRÍGUEZ et al., 2011; VAN DER PIJL, 1982).

There is good evidence that bats can discriminate, identify, and select VOCs (BIANCONI et al., 2007; LASKA, 1990a; MIKICH et al., 2003; PAROLIN; MIKICH; BIANCONI, 2015). However, the specific role of different molecular attributes in this process is largely unknown (HODGKISON et al., 2013; LASKA, 1990a, 1990b). Here, we study the molecular basis of fruit attraction in two neotropical fruit bat species, *Carollia perspicillata* and *Artibeus lituratus*.

METHODS

Oil "aging"

Crude oil (obtained through hydrodistillation, 2.08 mL of oil per 52 mL of diethyl ether) of *Piper gaudichaudianum* (Piperaceae) was impregnated in rubber septa (n=52, 8 mm of diameter) in a covered glass beaker for 30 minutes. All impregnated septa were kept under greenhouse condition until analysis or attraction trials, both conducted on the same day, every 5 days for up to 60 days (T_0 to T_{60}). Rubber septa were used as the receptacles for the essential oil because they release all the oil's components according to their boiling points. Moreover, adsorbed components are expected to remain intact until being released or chemically extracted.

CG-MS analysis

For GC-MS analysis, the remaining compounds of the essential oil were extracted from the septa (a triplicate) every 5 days with 2 mL of diethyl ether for 30 minutes in a sealed tube submitted to 5 minutes of sonication at the beginning of the procedure. The extract was then transferred to a vial, the solvent was evaporated until 1 mL and 25 μL of 5α-cholestane (10 μg/μL) was added for semi-quantification. All solutions were stored at -20°C until analysis. Samples were introduced via split/splitless injector (split 1:100, 290°C) equipped with a capillary column DB5 MS (60 x 0.25mm, 0.25 µm film thickness). The GC oven was programmed from 50 to 300°C at 3°C/min. Helium at a constant flow rate (1.0 ml/min) was the carrier gas. The GC-MS interface and ion source were 300°C and 200°C respectively. The ion trap mass spectrometer was operated in electron ionization mode at 70 eV, scanning m/z 50 - 650 (0.58 total scan time), and emission current 250 mA. Kovats retention index were calculated by injection of a standard saturated n-alkanes homologous series (C_7 – C_{30}) and, the structural identifications were performed by comparison of the fragmentation pattern and corresponding Kovats index with those reported in Adams (ADAMS, 2007).

Attraction trials

Ten adult Short-tailed fruit bats *Carollia perspicillata* (5 males and 5 females) and 10 adult Great fruit-eating bats *Artibeus lituratus* (5 males and 5 females) were captured from southern Brazil Atlantic Forest fragments. They were kept in a cage with two communicating rooms (2.8 m x 2.5 m x 2.8 m each) at the Natural History Museum of ‘Capão da Imbuia’, Curitiba – Parana (license No.14026-7 of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA) and fed with tropical fruits except during trials. Trials were conducted every five days during the night (T_0, T_5, T_{10}… T_{60}). An infrared video camera (SONY DCR-HC28 - PAROLIN; MIKICH; BIANCONI, 2015) was used to record the response of bats to the stimuli with minimum disturbance. Stimuli consisted of two septa, one with the essential oil of *Piper gaudichaudianum* impregnated at T_{0} – T_{60} (kept along those used for CG-MS analyzes) and one without it (control). These two septa were offered simultaneously, 90 cm apart, hanging from the cage ceiling by a 50 cm long nylon thread. Each trial followed five steps: 1) stimuli were arranged in one of the cage´s room (the position of the stimuli was switched between trials for randomization), 2) 10 individual bats of the same species were released in that room; 3) foraging attempts were recorded for 1 hour; 4) bats were removed from the room; 5) videos were watched later to enumerate the number of foraging attempts towards each septum (method "all occurrences"(ALTMANN, 1974)). All protocols were in accordance with recommendations from the American Society of Mammalogists (SIKES; GANNON, 2011) and were carried out under the control of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (No.14026-7).

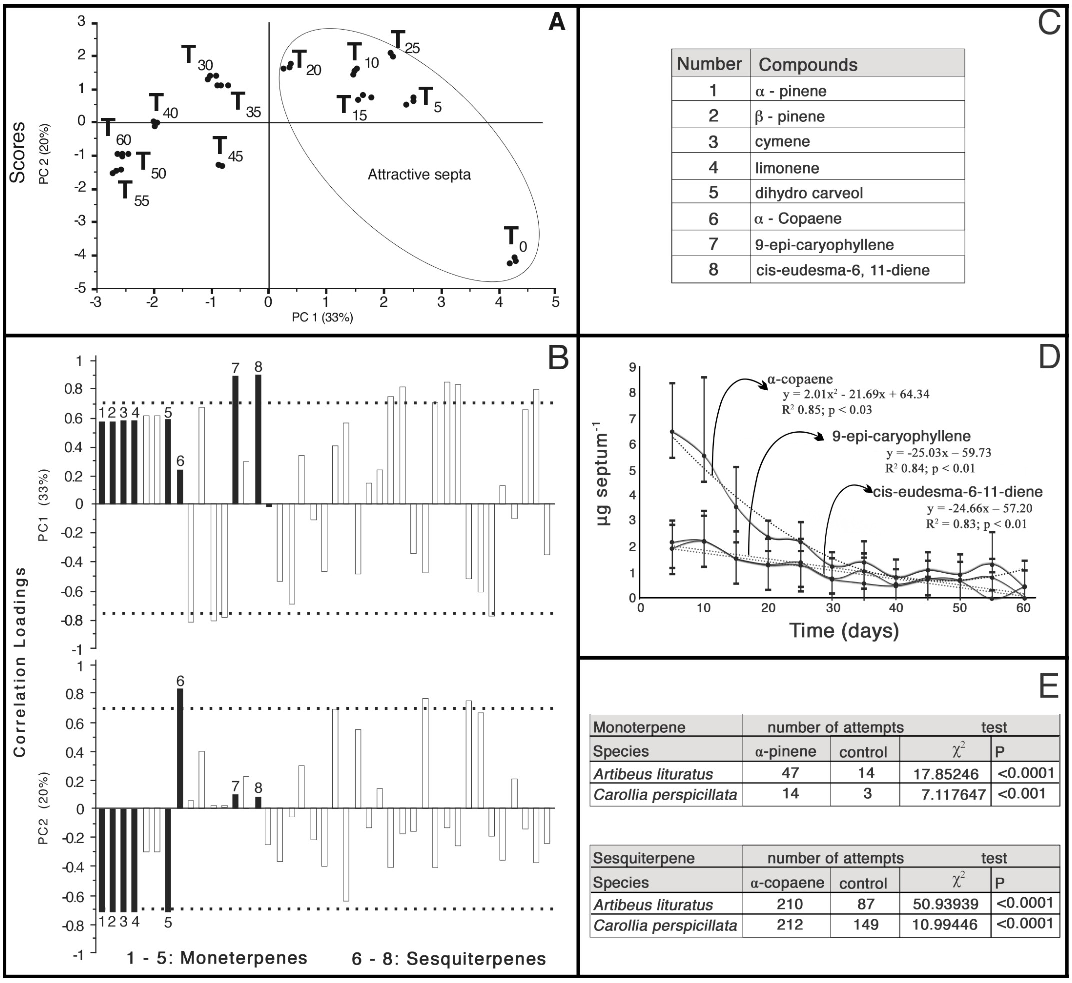
A PCA was applied to analyze similarities and differences in the oil composition in the septa over time. The PCA was run after -$\log_{10}$ transformation to compare the relative attraction of oil components within trials (T_0, T_5, T_{10}… T_{60}). Integrated chromatograms were normalized and each individual oil component was expressed as a relative amount (Ra, %) of the sum of all compounds detected (> 0.5%, total 100%). Only compounds detected in all triplicates were included in the analyses. In the absence of a specific compound at Tx, $\log_{10}$ (Ra) was taken as zero and included in the analysis. A chi-square test was used to compare the number of foraging attempts for each stimuli (oil *vs*. control) at significance level of 5%. Based on these results, additional trials (one for each compound and bat species) were conducted with two essential oil compounds: α-pinene and α-copaene (Sigma – Aldrich: 80605 and 27814, respectively) against a blank septum.

RESULTS

Captive *C. perspicillata* used in “oil aging” tests (see Methods) responded positively and significantly for up to 25 days (T_0 to T_{25}) to the gradual release of an essential oil extracted from a mature chiropterochoric fruit (Figure 1A). Principal components (PC1 and PC2) encompassed 53% of total variance of the essential oil data set. The first component (PC1) accounted for 33% of total variance and allowed the separation of attractive septa (T_0 to $T_{25,}$ positive values) from non-attractive septa (T_{30} to $T_{60,}$ negative values). Interestingly, PC2, which comprised 20% of the variance, demonstrate that the essential oil composition in T_0 is different from T_5 to T_{25} (Figure 1A).

The contribution of each remaining compound to the septa reflects the loading values derived by PCA. Compounds whose correlation loadings were >0.7 defined the PC. PC1, that describes attractive septa, was influenced most by 9-epi-caryophylene and cis-eudesma-5-11-diene (Figure 1B and C). PC2, that reveals the difference between T_0 and remaining attractive septa (T_5, T_{10}, T_{15}, T_{20} and T_{25}), was defined by α-copaene for positive values (T_5 to T_{25}) and α-pinene, β-pinene, cymene, limonene and dihydro carveol for negative values (T_0) (Figure 1A, B and C). The compounds α-pinene, β-pinene and limonene were detected exclusively in T_0, while cymene and dihydro carveol were detected up to T_5 and T_{10}, respectively. Other important compounds (Figure 1C, 1D), like α-copaene, 9-epi-caryophylene and cis-eudesma-6-11-diene, were detected at all times, up to T_{60} although their relative amount decreased over time. Interestingly, α-copaene went through a sharp decrease (polynomial equation) from T_5 to T_{25}, reaching a similar amount of 9-epi-caryophyllene and cis-eudesma-6-11-diene at T_{30}. During this period, the essential oil transformed from a mixture of highly volatile monoterpenes and sesquiterpenes to a more stable mixture containing only sesquiterpenes (Figure 1B).

When the standard compounds (α-pinene and α-copaene) were offered to the captive bats, both species invested significantly ($p < 0.01$) more time at the septa impregnated with the compounds in comparison with the control. Additionally, we observed approximately four times more feeding attempts with the sesquiterpene α-copaene than with the monoterpene α-pinene (Figure 1E).



Number	Compounds
1	α - pinene
2	β - pinene
3	cymene
4	limonene
5	dihydro carveol
6	α - Copaene
7	9-epi-caryophyllene
8	cis-eudesma-6, 11-diene

Monoterpene	number of attempts		test	
Species	α-pinene	control	χ^2	P
Artibeus lituratus	47	14	17.85246	<0.0001
Carollia perspicillata	14	3	7.117647	<0.001

Sesquiterpene	number of attempts		test	
Species	α-copaene	control	χ^2	P
Artibeus lituratus	210	87	50.93939	<0.0001
Carollia perspicillata	212	149	10.99446	<0.0001

Figure 1| (A) PCA analysis of GC-MS profiles of the essential oil of *Piper gaudichaudianum* remaining in rubber septa - the first two principal components clearly separated attractive septa for *Carollia perspicillata*, (B) correlation loadings of main chromatogram areas in terms of contribution to component 1 and 2 of figure A, (C) highlighted compounds of Figure B, (D) behavior of α-copaene, 9-epi-caryophylene and cis-eudesma-6-11-diene along the duration of the experiment (60 days), and (E) number of foraging attempts in 1 hour by ten individuals of captive fruit bat species (*A. lituratus* and *C. perspicillata*) on standard compounds (α-pinene and α-copaene) compared to a control.

Although it is well known that fruit bats are attracted to essential oils extracted from chiropterochoric fruit (BIANCONI et al., 2007; MIKICH et al., 2003), this is the first demonstration that they are actually attracted to the monoterpenes and sesquiterpenes released by these oils: initially as a mixture and later by sesquiterpenes alone. Among eight oil compounds (five monoterpenes and three sesquiterpenes) with prominent role in this interaction, α-copaene appears to play the key-role, since the concentration of this compound decreased sharply until the turning point in bat response (at T_{25}). Captivity tests confirmed that these fruit bat species are highly attracted to septa solely impregnated with this compound, confirming that the high volatility of the terpenes is a key signaling factor in the bat-plant interaction.

Captive fruit-eating bats were assumed to react to the semiochemicals released by the septa in a similar way to how they react to the chemical released by fruit. The synthesis and emission of essential oils increases with fruit maturation and becomes constant or decreases with fruit senescence (DUDAREVA et al., 2013; RANGANATHAN; BORGES, 2010). The release of scent is not only regulated by plant development, but also by environmental factors (e.g. ambient light/temperature) (DUDAREVA et al., 2006; YU; UTSUMI, 2009). Bats are induced to forage when sesquiterpenes concentrations are high. When their concentration falls below a minimum level (Figure 1D), the bats lose interest in the resource, probably because it is chemically signaling a decline in resource quality (fruit senescence).

Odor plumes emanate in a turbulent pattern away from their source (WILLIS, 2008). This means that the chemicals do not flow by diffusion, but follow a complex patchy structure after being released from the matrix (MURLIS; ELKINTON; CARDÉ, 1992; WILLIS, 2008). Animals such as fruit bats with well developed olfactory abilities may therefore be able to track resources using the flow direction of odor plumes carrying the target essential oil component released from the fruits. Mono- and sesquiterpenes are ubiquitous in plants (SEIGLER, 1998). Considering that VOCs (essential oil) signal the maturation stage (phenofase) of a fruit (RODRÍGUEZ et al., 2011; VAN DER PIJL, 1982), we suggest that these classes of terpenes are produced by plant at the same time (although with different biosynthesis pathways according to the plant species (MCGARVEY; CROTEAU, 1995; THOLL, 2006). Moreover, their physical properties regulate their emission from the mature fruit (i.e. boiling point and the vapor pressure). Taking into account that (1) monoterpenes were the first components released (Figure 1A and 1B), (2) that they are highly volatile (α-pinene boiling point: 155 °C), and (3) that bats were able to detect a single monoterpene component (Figure 1E), we propose that monoterpenes travelling within odor plumes are the first signal detected by foraging fruit bats. Once they have located the food source, the heavier and less volatile sesquiterpenes (α-copaene boiling point: 245 °C) signal the resource quality of the fruit and therefore maintain the interest of the bats for longer periods (Figure 1D). This mixture of light and heavy terpenes in essential oils therefore seems to regulate fruit finding and consumption and, by extension, the process of seed dispersal.

Our study was able to demonstrate that fruit bats can discriminate and select single fruit chemical compounds, which seem to have differential roles in bat-plant communication. Specifically, highly volatile short-lived monoterpenes attract bats to the food source and heavier, longer-lived sesquiterpenes signal which fruit are mature and ready to be eaten. These novel results have a clear practical significance. Essential oils are currently used to attract seed-dispersing bats to degraded areas as a way of facilitating natural regeneration via increased seed rain (BIANCONI et al., 2007, 2012; MIKICH et al., 2003). These oils are typically extracted from large amounts of wild fruit species in a costly and time-consuming process. Our results suggest that they could be replaced by a simple mixture of commercial monoterpenes and sesquiterpenes (e.g. α-pinene and α-copaene).

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CAPÍTULO 3 - DIET OF THE OLD WORLD FRUIT BATS: A REVIEW

ABSTRACT

Fruit bats of the family Pteropodidae are distributed in Africa, Asia, and Oceania including many Pacific islands. Although they are known as good dispersers, there is no broader assessment of the diversity of the diet of the genera (44 to 46) across different localities, to elucidate possible preferred resources, or to seek patterns in the bat-fruit interactions. To do that, we analyzed the frugivorous diet of the Pteropodidae family through an extensive bibliographical review, seeking to document patterns of consumption and also the possible congruence between the updated biogeographic realms proposed by HOLT et al. (2013) with bat-fruit relations in the Old World. The review resulted in 220 studies and 2,415 records of 33 bat genera consuming 601 species of 325 genera from 106 angiosperms families. The most consumed fruit genus was Ficus, in almost all zoogeographic realms. There is congruence between the realms and the bat-fruit relationship, evidencing similarities and consistencies related to the group's adaptive radiation. The data from this study confirmed the great diversity in the diet of this family and also showed a pattern of frugivorous consumption with a preference for the genus Ficus, both among most flying fox genera and across the zoogeographic realms. Data of this nature help to visualize patterns and similarities among different regions of the globe and different animal groups, and can be used as a background for inferences related to different ecology and biology areas.

Key words: Chiroptera, *Ficus*, frugivory, Pteropodidae, zoogeography

INTRODUCTION

Old World fruit bats are members of the family Pteropodidae, distributed throughout the tropical regions of Africa, Asia, and Oceania including many Pacific islands (IUCN, 2016; SIMMONS, 2005). There are currently 198 recognized species in between 42 and 46 genera (ALMEIDA et al., 2011; FENTON; SIMMONS, 2014; SIMMONS, 2005). They are characterized by their often large size, with forearm length ranging from 42 to 200 mm; the largest species are found in the genera commonly referred to as flying foxes, *Pteropus* Brisson, 1762 and *Acerodon* Jourdan, 1837 (ALMEIDA et al., 2011; NOWAK, 1999; SIMMONS, 2005).

Based upon molecular studies, the Pteropodidae, along with the other groups within Yinpterochiroptera, first appeared in Asia (ALTRINGHAM, 2011; TEELING et al., 2005). Despite the scarce fossil evidence (GUNNELL; SIMMONS, 2005), phylogenetic analyses point to an explosive diversification of Chiroptera in the Eocene, separating Pteropodidae from other groups approximately 52 to 50 million years ago (ALMEIDA et al., 2011; TEELING et al., 2005), following the great diversification of angiosperms (DAVIES et al., 2004). The radiation of the megabat crown group occurred between 31 and 20 million years ago (ALMEIDA et al., 2011). The ecological opportunity (see Simpson 1953) involved in this radiation may be related to the extinction of competitive groups, demographic changes, or key innovations such as the diversification of body size and emergence of phytophagous foraging habits (YODER et al., 2010).

These plant-visiting bats are considered generalists, consuming fruits, nectar, pollen, and even leaves; they interact with many species of plants of importance to people (FUJITA; TUTTLE, 1991; MARSHALL, 1983). Their role in seed dispersal and pollination has both positive economic and ecological impacts especially in promoting forest recovery through the increase of seed rain and seed bank or the dispersal of plants to recent oceanic islands as was the case on Krakatau in Indonesia (DOCTERS VAN LEEUWEN, 1935; MUSCARELLA; FLEMING, 2007; WHITTAKER; JONES, 1994).

The frugivorous diet of pteropodids is documented mainly by direct observations, seed collection, and pellets under the feeding roost (VOIGT et al., 2009). In general, megabat diet studies are limited to consumption records of a particular species, or of some species at a given location (e.g. Banack 1998; Picot et al. 2007; Mahmood-Ul-Hassan et al. 2010; Andrianaivoarivelo et al. 2011; Sudhakaran and Doss 2012), with few articles dealing with a more comprehensive scenario, taking into account the diversity of the group and its total geographic distribution (e.g. Fujita and Tuttle 1991). There is no broader assessment of the diversity of the diet of the genera across different localities, to elucidate possible preferred resources, or to seek patterns in presence or absence of the bat-plant interactions.

We were interested in observing patterns of bat-plant ecological interactions across the various major biomes where pteropodids occur. Recently, Holt et al. (2013) published an update of the six zoogeographic regions of Wallace, dividing the planet into 11 realms and 20 different zoogeographic regions. These maps were generated from distribution data and phylogenetic relationships of the world's amphibians, non-pelagic birds and non-marine mammals (HOLT et al., 2013).

Based on this idea, we analyzed the frugivorous diet of the Pteropodidae family through an extensive bibliographical review, seeking to document patterns of consumption and the possible congruence between the biogeographic realms of Holt et al. (2013) with bat-fruit relations in the Old World.

METHODS

The study consisted of a literature review and data compilation of fruit consumption by representatives of the family Pteropodidae throughout their distributional range. We performed an exhaustive search in Web of Science (http://apps.webofknowledge.com) with the following keywords and combinations: "flying foxes", "Pteropodidae”, “old World fruit bat”, "Megachiroptera", “Yinpterochiroptera”, "Chiroptera", "bat", "diet", "fruit", "frugivory", “frugivorous”, “dispersal”, “seed” and "plant”. All consumption fruits data found were recorded on a spreadsheet, excluding those without any reference of locality for the dietary record. Prior analyses, duplicate information derived from compilations of primary data sources (i.e. review articles, studies referring to data collected and published in other sources), were excluded. Secondary data were only used in few cases if the cited study could not be found.

The collected information was organized in a spreadsheet with the following entries: bat (species and/or genus), fruit consumed (species, genus and/or family), location, recording method and source. For the distribution of points by zoogeographic realms (HOLT et al., 2013), the information of longitude and latitude of the consumption points were taken from the data source (when available) or from Google Earth, based on information provided by the authors. Due this lack of geographical information, in some cases, the points of the consumption records are slightly outside the distribution area of the flying foxes (Figure 1A). Each entry of a plant species (genus or family when unknown species) accounted for one record. Records could be based on direct observations, analysis of stomach contents and/or analysis of fecal samples.

The data were first tabulated as cited in the source and the taxonomy subsequently standardized according to Simmons (2005) for bats, with the exception of the genera *Desmalopex* Miller, 1907 which was maintained as named by the authors (ESSELSTYN et al., 2008). Plant species names were standardized based upon The Plant List (THE PLANT LIST, 2013) for the plant species included in the spreadsheet. To analyze the data using the update published by Holt et al. (2013), we used the shape files provided by the authors (http://macroecology.ku.dk/resources/wallace) to separate the diet records and to build the maps. Only the data on the realms were included in maps and analysis (Afrotropical, Australian, Madagascan, Nearctic, Neotropical, Oceanian, Oriental, Palearctic, Panamanian, Saharo-Arabian and Sino-Japanese).

We used two procedures to compare the composition of dietary fruit species/genera across zoogeographic realms. Based upon records we included eight zoogeographic realms in the analysis: Saharo-Arabian, Afrotropical, Madagascan, Palearctic, Sino-Japanese, Oriental, Australian and Oceanian. Due to the small number of observations for the Palearctic realm, we merged those observations with the adjacent Sino-Japanese, since the localities for the Palearctic were also near the division between these two realms. This left us with seven remaining realms. We ran an ordination on the remaining data set using Non-Metric Multidimensional Scaling (NMDS) with zoogeographic realms as the sites and fruit observations as the “species” list. The NMDS was run in NMS Autopilot mode in PC-ORD version 5 (MCCUNE; GRACE; URBAN, 2002). Autopilot mode conducts multiple runs and selects the best solution at each dimensionality for generating the final outcome. Data were input using Sørensen (Bray-Curtis) distance. We also ran a nearest neighbor cluster on the Relative Sørensen distance measure (FAITH; MINCHIN; BELBIN, 1987) to gain additional insights on the relationships among zoogeographic realms. The program Quantum GIS was used to build the maps (QUANTUM GIS DEVELOPMENT TEAM, 2015), using Holt et al. (2013) for the zoogeographic realms and IUCN (2014) for the distribution of bat species.

RESULTS

The literature review resulted in 220 studies and 2,415 records of fruit consumption by the 33 bat genera. Altogether, we recorded 601 species of 325 genera from 106 botanical families. Of these, 1,925 were identified to the species level, 478 only to genus, and the remainder (n = 12) to family. A total of 92 bat species were documented with some record of fruit consumption, with 2,369 records identified to bat species and 45 only to genus. A listing of the bats and the number of records for each is presented in Appendix 1.

The bat genera with the most records were *Pteropus* (n = 1,273) with 34 species, *Cynopterus* (n = 355) with 4 species and *Rousettus* (n = 245) with 5 species, with the genus *Pteropus* responsible for over half of all records (52,7%). The genera of fruit consumed by bats varied by genus of bat and by zoogeographic realm (Table 1).

Table 1. The most consumed fruit genera per flying fox and per zoogeographic realms. Up to three fruit genera with the major number of records are represented, followed by its record number (n). Ngen= number of fruit genera; Nrec= total number of records.

Bat genera	**Ngen**	**Nrec**	**Most common fruit genera**	**Realms**
Acerodon Jourdan, 1837	2	10	*Ficus* (n=9); *Nauclea* (n=1)	Oriental
Aproteles Menzies, 1977	1	2	*Ficus* (n=2)	Oceanian
Balionycteris Matschie, 1899	14	24	*Ficus* (n=6); *Eugenia* (n=2); *Fragraea* (n=2)	Oriental
Chironax K. Andersen, 1912	5	9	*Ficus* (n=5); *Adinandra* (n=1); *Diospyros* (n=1)	Oriental
Cynopterus F. Cuvier, 1824	95	355	*Ficus* (n=85); *Psidium* (n=17) *Mangifera* (n=13)	Oriental
Desmalopex Miller, 1907	2	3	*Ficus* (n=1); Melastomataceae (n=1); *Musa* (n=1)	Oriental
Dobsonia Palmer, 1898	14	23	*Ficus* (n=6); *Carica* (n=3); *Musa* (n=3)	Australian (*Ficus*, n=1); Oceanian (*Ficus*, n=4; *Carica*, n= *Musa*, n=3); Oriental (*Areca*, n=1; *Borassus*, n=1; *Fic* n=1)
Dyacopterus K. Andersen, 1912	3	6	*Ficus* (n=4); *Elaeocarpus* (n=1); *Prunus* (n=1)	Oriental
Eidolon Rafinesque, 1815	54	142	*Ficus* (n=25); *Psidium* (n=10); *Mangifera* (n=8)	Afrotropical (*Ficus*, n=15; *Mangifera*, n=8; *Musa*, n= Madagascan (*Ficus*, n=8; *Psidium*, n=3; *Solanum*, n= Saharo-Arabian (*Ficus*, n=2; *Phoenix*, n=1)
Eonycteris Dobson, 1873	1	1	*Palaquium* (n=1)	Oriental
Epomophorus Bennett, 1835	40	133	*Ficus* (n=28); *Mangifera* (n=9); *Musa* (n=6)	Afrotropical (*Ficus*, n=27; *Mangifera*, n=9; *Musa*, n= Saharo-Arabian (*Ficus*, n=1)
Epomops Gray, 1870	19	37	*Ficus* (n=7); *Solanum* (n=5); *Musa* (n=4)	Afrotropical
Haplonycteris Lawrence, 1939	1	2	*Ficus* (n=2)	Oriental
Harpyionycteris Thomas, 1896	1	2	*Ficus* (n=1); *Freycinetia* (n=1)	Oriental
Hypsignathus H. Allen, 1861	11	24	*Ficus* (n=5); *Anthocleista* (n=3); *Mangifera* (n=3)	Afrotropical
Latidens Thonglongya, 1972	4	11	*Ficus* (n=6); *Elaeocarpus* (n=2); *Prunus* (n=2)	Oriental
Lissonycteris K. Andersen, 1912	8	11	*Solanum* (n=3); *Ficus* (n=2); *Adenia* (n=1)	Afrotropical
Macroglossus F. Cuvier, 1824	1	1	*Eugenia* (n=1)	Oceanian
Megaerops Peters, 1865	5	7	*Ficus* (n=2); *Palaquium* (n=2); *Elaeocarpus* (n=1)	Oriental
Melonycteris Dobson, 1877	1	2	*Ficus* (n=2)	Oceanian
Micropteropus Matschie, 1899	10	21	*Ficus* (n=6); *Annona* (n=3); *Psidium* (n=3)	Afrotropical
Myonycteris Matschie, 1899	4	7	*Solanum* (n=3); *Adenia* (n=2); *Ficus* (n=1)	Afrotropical
Nanonycteris Matschie, 1899	4	4	*Carica* (n=1); *Ficus* (n=1); *Milicia* (n=1)	Afrotropical
Nyctimene Borkhausen, 1797	6	14	*Ficus* (n=7); *Psidium* (n=2); *Randia* (n=2)	Australian (*Ficus*, n=7; *Psidium*, n=2; *Randia*, n= Oceanian (*Ficus*, n=3; *Casuarina*, n=1)
Otopteropus Kock, 1969	1	1	Ficus (n=1)	Oriental

Paranyctimene Tate, 1942	2	2	*Ficus* (n=1); *Piper* (n=1)	Oceanian
Ptenochirus Peters, 1861	9	19	*Ficus* (n=11); *Bischofia* (n=1); *Ceiba* (n=1)	Oriental
Pteralopex Thomas, 1888	13	16	*Ficus* (n=3); *Canarium* (n=2); *Annona* (n=1)	Oceanian
Pteropus Brisson, 1762	231	1,273	*Ficus* (n=230); *Mangifera* (n=51); *Terminalia* (n=49)	Afrotropical (*Ficus*, n=6; *Artocarpus*, n=3; *Mangifera*, n= Australian (*Ficus*, n=64; *Syzygium*, n=18; *Citrus*, n=1 Madagascan (*Ficus*, n=13; *Psidium*, n=5; *Mangifera*, n= Oceanian (*Ficus*, n=59; *Artocarpus*, n=36; *Termina* n=26); Oriental (*Ficus*, n=68; *Mangifera*, n=14; *Psidiu* n=14); Saharo-Arabian (*Ficus*, n=7; *Psidium*, n *Diospyros*, n=2); Sino-Japanese (*Ficus*, n=13; *Morus*, n *Musa*, n=3)
Rousettus Gray, 1821	66	245	*Ficus* (n=60); *Eriobotrya* (n=10); *Melia* (n=10)	Afrotropical (*Ficus*, n=7; *Eriobotrya*, n=4; *Diospyros*, n= Madagascan (*Ficus*, n=11; *Carica*, n=1; *Musa*, n= Oceanian (*Ficus*, n=1); Oriental (*Ficus*, n=19; *Psidium*, n *Mangifera*, n=5); Palearctic (*Prunus*, n=3; *Ficus*, n=2; *Me* n=1); Saharo-Arabian (*Ficus*, n=20; *Ceratonia*, n=8; *Me* n=6)
Scotonycteris Matschie, 1894	2	3	*Solanum* (n=2); *Ficus* (n=2)	Afrotropical
Syconycteris Matschie, 1899	5	6	*Ficus* (n=2); Moraceae (n=1); *Piper* (n=1)	Australian (*Ficus*, n=2; *Piper*, n=1); Oceanian (Morace n=1; Piperaceae, n=1; Solanaceae, n=1)
Thoopterus Matschie, 1899	1	1	*Ficus* (n=1)	Oriental

The plant genera most consumed by bats was *Ficus* L. (n = 519), followed by *Psidium* L. (n = 98) and *Mangifera* L. (n = 96), both of the latter with a much smaller number of records. Analyzing the data by bat genera, the pattern repeats most, with *Ficus* being the fruit with the largest number of records (Table 1). Only the genera *Macroglossus*, *Syconycteris* (both in the nectar feeding subfamily Macroglossinae) *Dobsonia*, *Harpyionycteris* (Harpyionycterinae), *Eonycteris* (Rousettinae), *Lissonycteris* (Myonycterini), *Megaerops* (Cynopterinae) and *Nanonycteris* (Epomophorinae) did not show this pattern, but all of these genera had a very small number of records. In general, the genus *Ficus* was also the most consumed in almost all the realms, representing 18.6% in Afrotropical (n = 78), 21.13% in Australian (n = 71), 23.52% in Madagascan (n = 32), 13.73% in Oceanian (n = 75), 26.42% in Oriental (n = 218), Saharo-Arabian (n = 93) and 27.65% Japanese (n = 13). The only exception was the Palearctic realm, with most of the records (21.42%) for *Prunus* L. (n = 3), however there were only 14 records for this realm.

We compiled records by zoogeographic realm and the number of consumed fruit genera consumed according to the proposal of Holt et al. (2013) (Table 2).

Table 2. Number of recorded feeding events and different fruit genera consumed by megabats in each zoogeographic realm (Holt et al. 2015). Note that the results for the Palearctic and the Sino-Japanese realms are pooled due to the very small number of Palearctic observations.

Zoogeographic Realm	**Observations**	**Genera**
Afrotropical	418	81
Australian	336	89
Madagascan	136	63
Oceanian	546	114
Oriental	825	142
Saharo-Arabian	93	22
Sino-Japanese/Palearctic	60	28

The Figure 1 illustrates the distribution of bats (Figure 1A), the consumption records and the zoogeographic realms (Figure 1B).

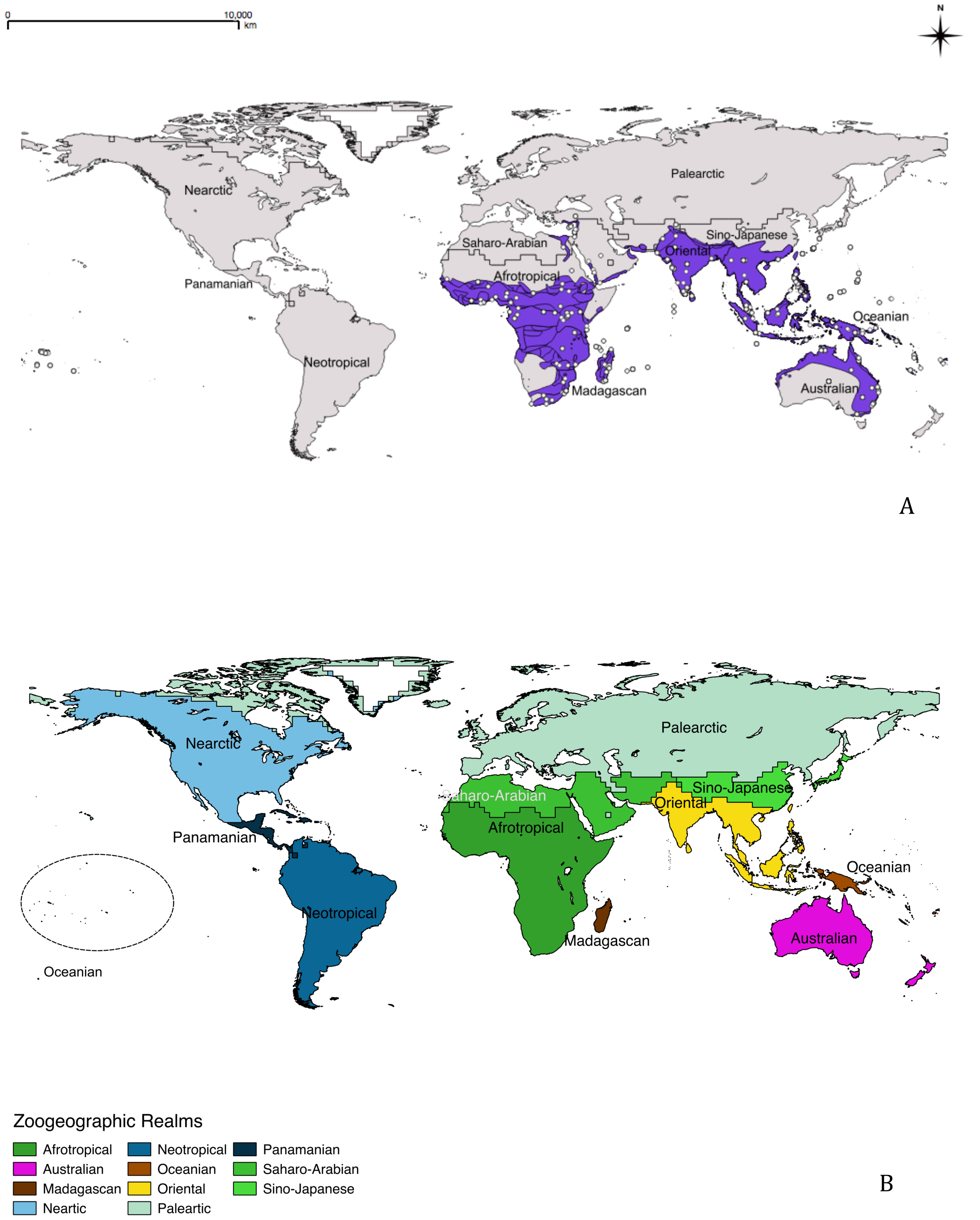

Figure 1. Flying foxes distribution (purple) and the localities with diet record found in this study (white circles). Zoogeographic realms according to Holt et al. (2013) .

The results of the NMDS show a great dispersion of zoogeographic realms by fruit consumption (Figure 2). Examining the loadings of the genera on the NMDS axes reveals associations among certain genera and the zoogeographic realms

(Appendix S2). Three zoogeographic realms show marked dispersion in the composition of the diet of fruit bats. The more northerly Sino-Japanese/Palearctic is highly distinctive; and an examination of fruits consumed show a preponderance of more temperate zone fruits (*Ampelopsis* Michx., *Ardisia* Gaertn., *Eurya* Thunb. and *Pieris* D. Don). Mediterranean species like *Ceratonia* L. and *Arbutus* L. are typical of the Saharo-Arabian realm. The Madagascanis also highly distinctive and dominated by regional and Malagasy endemics (e.g. *Anthocleista* Afzel.ex R.Br. *Brexia* Noronha ex Thouars, *Bakerella* Tiegh and *Sarcolaena* Thouars).

The remaining four realms are more clustered together and dominated by very high richness of a more tropical selection of fruit genera. Australian and Afrotropical realms are more dispersed than a tighter cluster of fruit species associated with the Oceanian and Oriental realms (Figure 2).

The cluster analysis (Figure 3) shows a pattern of similarity between the two more temperate regions and a strong cluster of tropical regions, with the closest association being between Australian and the Oriental realms, which are contiguous and with a historically close contact with the Sunda Plate. Oceanian clusters with the other easterly tropical sites, though is less closely related in the composition of fruits selected by pteropodids.

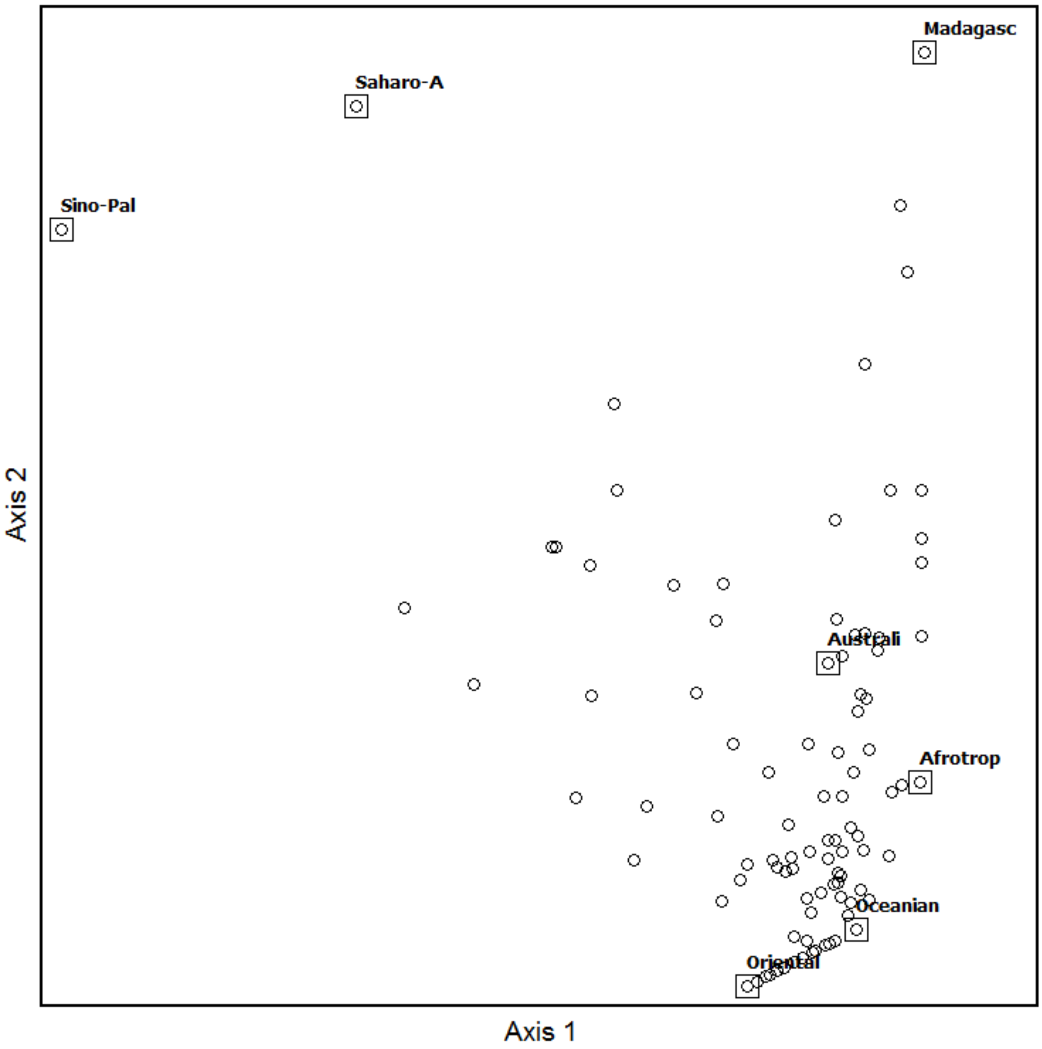

Figure 2. Ordination analysis by Non-Metric Multidimensional Scaling (NMDS) of realms by fruit present on frugivorous Pteropodidae diet. Abbreviations: Afrotrop – Afrotropical; Australi – Australian; Madagasc – Madagascan; Saharo-A – Saharo-Arabian; Sino-Pal – Sino-Paleartic.

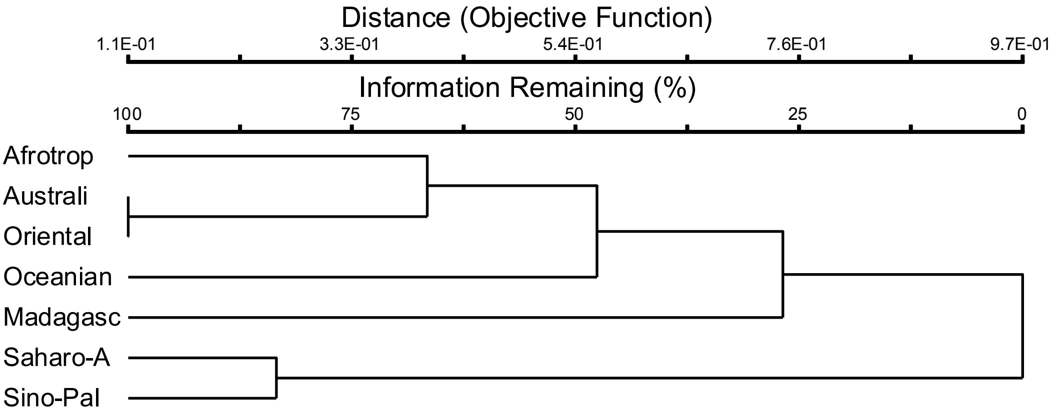

Figure 3. Cluster analysis using a relative Sørenson index of realms by fruit present on frugivorous Pteropodidae diet. Abbreviations: Afrotrop – Afrotropical; Australi – Australian; Madagasc – Madagascan; Saharo-A – Saharo-Arabian; Sino-Pal – Sino-Paleartic.

DISCUSSION

Our analysis of the frugivorous diet of flying foxes revealed great diversity in the diet, but also a consistent pattern of consumption of *Ficus* fruits by the majority of bat species across the different zoogeographic realms. In addition, there is congruence between the realms updated by Holt et al. (2013) and the bat-fruit relationship, evidencing similarities and consistencies related to the group's adaptive radiation.

The literature review resulted in data on the frugivorous diet for 33 of more than 40 genera of Pteropodidae (ALMEIDA et al., 2011; SIMMONS, 2005). The genus with the highest number of consumption records was *Pteropus*, corresponding to more than 50% of all the records. This genus has the largest number of species - approximately 65 - mostly distributed in the Pacific islands (FLEMING, 1993; SIMMONS, 2005). *Cynopterus* and *Rousettus* are the genera with the next most records, with the former restricted to the Oriental realm and the latter in all the Old World realms with the exception of the Paleartic and Australian (HOLT et al., 2013; IUCN, 2016; SIMMONS, 2005). The genera *Eonycteris, Macroglossus, Melonycteris* and *Syconycteris* are morphologically adapted to nectarivory (FLEMING; GEISELMAN; KRESS, 2009), but although nectar is their main food resource, they can also feed on fruits (e.g. Law 2001; Shanahan et al. 2001; Bumrungsri et al. 2013). Several of the genera have few records of consumption, with few published studies on the frugivorous fraction of their diet, for example *Thoopterus*, endemic to Sulawesi (Oriental realm), for which only one record of fruit consumption was found (MICKLEBURGH; HUTSON; RACEY, 1992).

In all, we found consumption records of 325 genera of plants within 106 botanical families. Flying foxes are recognized as generalists, consuming a diversity of fruits (MARSHALL, 1985), attributed to less predictability in resources and a sparse distribution of these resources in the Old World (DUMONT, 2003). This could explain the large number of records found for cultivated species (e.g. *Carica* L., *Mangifera*, *Musa* L., *Psidium* and *Vitis* L.) and their notoriety as a crop pest. Marshall (1983) treats them as "sequential specialists", since they select a range of fruits among those available in each period of time. However, according to Andrianaivoarivelo et al. (2013), they prefer native fruits when available. Their choices are directed to canopy fruits, with little consumption of understory plants (HENRY et al., 2004). Some authors explain this as a result of the low fruit diversity at these sites (compared to the Neotropics, for example) and their feeding mode, in which the animal lands on the plant - making it difficult to consume smaller herbaceous fruits (ALTRINGHAM, 2011; DUMONT, 2003; FLEMING, 1993), especially due to their larger size compared to microbats that in spite of their smaller size usually take fruits on flight.

Our data show that there are many fruit genera with few records in the diet and only a few highly utilized genera, both by genus of bat and by zoogeographic realm, especially highlighting the genus *Ficus*. Figs have a big-bang strategy and also intraspecifically asynchronous, serving as a food resource of great importance for several species of vertebrates, particularly mammals and birds (SHANAHAN et al., 2001b), an observation seems to hold in the Old World as well. We observed 77 of the species (31 genera) feeding on figs. This consistent diet pattern is even more pronounced than that observed for the less generalist Neotropical bats, where the so-called "preferential" fruit varies among bat genera (PAROLIN; BIANCONI; MIKICH, 2016). It is possible that even the patterns of use of space by pteropodids are related to the availability of *Ficus* fruits, driving ecological interactions based upon this important supply of nutrients and the high quantity of fruits available during fruiting, possibly contributing to the colonization of oceanic islands by *Ficus* many miles from the continent through the dispersal of seeds carried by bat vectors (BUSH; WHITTAKER; PARTOMIHARDJO, 1995; DUMONT, 2003; THORNTON; COMPTON; WILSON, 1996; WHITTAKER; JONES, 1994).

Updated zoogeographic realms vs. Bat diet

We used the new proposal of zoogeographic realms proposed by Holt et al. (2013) to examine patterns of fruit consumption by Old World pteropodids. Factors that determine the distribution of an animal or plant group include geological history, climate, food resource availability, chemical environmental factors, and competition, for example (COX; MOORE, 2009). According to Sanmartín and Ronquist (2004), the distribution pattern of plants in the southern hemisphere is better explained by events of extinction and dispersion than by the breakdown of the Gondwana continent itself. De Queiroz (2005) further states that oceanic dispersion has a greater influence on the current distribution of taxa than vicariance. Thus, to explain the similarities presented in the plants consumed by bats, one must consider the configuration of the planet at the beginning of the Cretaceous, a period of great diversification of angiosperms (CRANE; FRIIS; PEDERSEN, 1995; CRANE; LIDGARD, 1989), in which the territories belonging today to the zoogeographic realms were united (Sino-Japanese and Paleartic) or still relatively close (Afrotropical, Australian, Madagascan, Oceanian and part of Oriental) (see Cox and Moore 2009). When we observe the clusters of fruit consumption formed with the Afrotropical, Australian, Oceanian and Oriental realms, for example, with several genera of fruits in common, such as *Anacardium* L., *Annona* L., *Litchi* Sonn., *Morinda* L. and *Parinari* Aubl. it is evident that this is a reflection of the influence of Gondwana biogeography.

We see a similar pattern for Sino-Japanese and Paleartic, which possess a low number of records and share close geographic proximity and were lumped together for the analyses. Typical genera include *Citrus* L., *Ficus*, *Prunus*, *Diospyros* L. and *Morus* L.. The Saharo-Arabian realm has a smaller distribution of angiosperms and bats based upon climatic transitions during the Lower Pleistocene resulting in the desertification in this region (COX; MOORE, 2009). The area is also considered by Morrone (2015) as a transition zone between the Paleartic and Ethiopian (here treated as Afrotropical), resulting in the clustering of that realm to Sino-Paleartic. The fruits tied to this Sino-Paleartic junction are: *Alpinia* L., *Ampelopsis, Ardisia, Eurya, Ginkgo* L. and *Pieris*, all with records of consumption from the islands of Japan (FUNAKOSHI; WATANABE; KUNISAKI, 1993; MICKLEBURGH; HUTSON; RACEY, 1992). The key genera for Saharo-Arabian were recorded for Israel and the island of Cyprus, with some native (*Arbutus, Ceratonia, Crataegus* L.*, Styrax* L.) and others introduced (*Lantana* L. and *Washingtonia* H. Wendl.) (DEL VAGLIO et al., 2011; KORINE; IZHAKI; ARAD, 1999).

The Oriental region has the highest number of registries, with almost 35% of the total. This large number of bat-plant interactions can be related to the fact that this region is the point of origin of flying foxes (TEELING et al., 2005). Although the geographic origin of the group has been questioned due to the limitations in the fossil record (MARSHALL, 1983), a little more than a decade ago Teeling et al. (2005) published a robust evolutionary tree of the Chiroptera which points to the Asian continent as the initial site of the Pteropodidae radiation. According to Fleming et al. (1987), Southeast Asia has almost twice the number of species of fruit bats per area compared to the Neotropics and West Africa. Some of the genera observed in the diet of pteropodids (e.g. *Adinandra* Jack, *Chrysophyllum* L., *Lannea* A. Rich. in

Guillem., *Payena* A. DC. and *Xerospermum* Blume) do not have consumption records in the other realms, however are not endemic to the Oriental. Given the high species richness of pteropodids in this region, we might expect more partitioning of the available resource base, and potentially more specialization in food resources among the many genera and species of bats.

The Madagascan region is an interesting case apart. The separation of diet composition for Malagasy bats from other groups in the ordination is easily explained by the extreme endemism of Madagascar, where more than 80% of the plants are endemic (Myers et al., 2000; Yoder and Nowak 2006). This high degree of endemism is related to the long period of isolation; Madagascar and India separated from the African continent 135 million years ago, and India then separated from Madagascar 88 mya (Storey et al., 1995). Although the genera of Yinpterochiroptera present in Madagascar are not endemic to the island, several of the genera of fruits consumed by them are, such as *Canephora* Juss., *Cinnamosma* Baill. *Sarcolaena* and *Tambourissa* Sonn. (MISSOURI BOTANICAL GARDEN, 2016). However, the connection with the other realms in the cluster (except for Sino-Paleartic and Saharo-Arabian - see Figure 3), although initially related to the biogeographic scenario of Gondwanaland 100 million years ago (COX; MOORE, 2009) is weak, related to the long history of isolation and the high levels of endemism evolved. Studies relate the dispersion of Cenozoic fauna of African origin as the most important "biogeographic force" in modeling the fauna and flora of today's Madagascar (SANMARTÍN; RONQUIST, 2004; YODER; NOWAK, 2006).

The family Pteropodidae is well studied in several aspects of its ecology, but this is the first compilation of data on the group's frugivorous diet throughout its distribution. The data from this study confirmed the great diversity in the diet of this family and also showed a pattern of frugivorous consumption with a preference for the genus *Ficus,* both among most flying fox genera and across the zoogeographic realms. In addition, it was possible to use these Old World bat-plant interactions to corroborate the new zoogeographic realms proposed by Holt et al. (2013) based upon the patterns of frugivory. Data of this nature help to visualize patterns and similarities among different regions of the globe and different animal groups, and can be used as a background for inferences related to biogeography, ethology, botany and ecology.

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CAPÍTULO 4 - FRUGIVOROUS BATS AS FACILITATORS OF NATURAL REGENERATION IN DEGRADED HABITATS: A GLOBAL TOOL

ABSTRACT

Fruit bats are important seed dispersers in the Tropical World. Their keen sense of smell has led to the development of a restoration tool that uses the essential oils of their preferred fruit species as decoy, attracting bats to degraded areas and increasing local seed rain. Although this technique has been based on Neotropical frugivorous phyllostomids - using the genera *Artibeus*, *Carollia* and *Sturnira* as models - its use is not expected to be limited to this region. So, the objective of this study was to draw parallels between seed dispersing Yinpterochiroptera and Yangochiroptera that would allow the application of the same technique in the Old World. As a starting point, an extensive literature review was performed on diet, fruit consumption and seed dispersal by flying foxes (Pteropodidae). Data revealed that *Cynopterus*, *Pteropus* and *Rousettus,* due to their wide distribution, large diet diversity and great consumption of *Ficus* - whose oils already proved to attract phyllostomids - seem to be the closest functional genera to the Phyllostomidae used as models in the New World. So, now captive and field experiments must be designed to further develop the use of essential oils to attract Old World bats and contribute with the restoration of degraded forests and their own conservation since several pteropodids are endangered worldwide.

Key words: Chiroptera, diet, essential oil, *Ficus*, Phyllostomidae, Pteropodidae, olfaction, restoration technique, seed dispersal, seed rain

INTRODUCTION

Phytophagous bats play an important role in pollination and dispersal in tropical forests (FLEMING, 1988; MARSHALL, 1983). Representatives of the family Pteropodidae (Yinpterochiroptera) are distributed in Asia, Africa and also in Oceania and have hundreds of species of plants in their diet, contributing to colonization (and recolonization) of plants in the tropics, including islands and archipelagoes of the Pacific (COX et al., 1991; FUJITA; TUTTLE, 1991). The New World phytophagous bats of the family Phyllostomidae, distributed all over the Neotropical region

(SIMMONS, 2005), are also known to use more than 700 species of flowers, fruits and leaves as food resources (BREDT; UIEDA; PEDRO, 2012).

The phyllostomid genera *Artibeus* Leach, 1821, *Carollia* Gray, 1838 and *Sturnira* Gray, 1842 are widely distributed in the Neotropics and are the most cited bat species in animal-fruit studies (ANDRADE et al., 2013; CHARLES-DOMINIQUE, 1986; FLEMING, 1988; GARDNER, 2007; GIANNINI, 1999; THIES; KALKO; SCHNITZLER, 1998). Together, these three genera are known to feed on 176 genera of fruit (PAROLIN; BIANCONI; MIKICH, 2016). Nevertheless, a few genera dominate their diet and there are strong and consistent associations of the genera *Artibeus* with *Ficus* L. (Moraceae), *Carollia* with *Piper* L. (Piperaceae) and *Sturnira* with *Solanum* L. (Solanaceae) (BONACCORSO, 1979; CHARLES-DOMINIQUE, 1986; FLEMING, 1988; IUDICA; BONACCORSO, 1997; KALKO; HANDLEY-JR; HANDLEY, 1996; MELLO; KALKO; SILVA, 2008; PALMEIRIM; GORCHOY; STOLESON, 1989; WENDELN; RUNKLE; KALKO, 2000). A recent review (PAROLIN; BIANCONI; MIKICH, 2016) even revealed that these preferences are consistent along the time (annual cycle) and space (whole Neotropical region) so that whenever and wherever these fruit are available they are preferably consumed by these bats.

Studies focusing on the potential of essential oils to attract fruit-eating bats found that the oil of preferred chiropterochoric species was effective in attracting bats both within the forest (MIKICH et al., 2003) and in open areas (BIANCONI et al., 2007). These results led to the proposal of a restoration tool to be used in degraded forest areas based on the improvement of seed rain and seed bank due to the increased activity of fruit bats attracted by essential oils extracted from chiropterocoric fruits (BIANCONI et al., 2007). This decoy has been tested in the

Neotropical region and results show that the activity of seed dispersing bats do increase around the odorous stimuli (BIANCONI et al., 2012).

A tool capable of mediating the dispersal of forest seeds to degraded areas is particularly important given the current conservation status of tropical regions, especially considering the decrease in natural areas. Deforestation reached 129 million hectares around the planet in 2015, according to the Food and Agriculture Organization of the United Nations (FAO, 2015). Current efforts and traditional techniques do not seem enough to cope with this huge demand. Besides, it is important to restore not only the function, but also the form of natural forests and most restoration techniques do not include the diversity of plant and life forms usually consumed and dispersed by animals (CHAZDON, 2008).

Although this technique has been developed in the Neotropics, it is not necessarily restricted to this region. Old World seed dispersers do not echolocate (except for *Rousettus* Gray, 1821) and have a better vision than microchiropteran frugivores (ALTRINGHAM, 2011), but more importantly, both groups have a well-developed sense of smell used to find and select ripe fruit (FLEMING, 1988; HODGKISON et al., 2013).

Based on this information, the objective of this study is to evaluate the potential for expansion of this restoration tool for degraded areas including the attraction of Old World fruit bats. Following the work already done with the phyllostomid *Artibeus*, *Carollia* and *Sturnira* in the Neotropics, we wish to find flying-fox genera that could serve as basis for the implementation of the technique. The goals are to facilitate the development of strategies, enable adjustments to the design, and procure the selection of fruit species of the Old World for the extraction and fractionation of essential oils.

METHODS

We conducted a literature review and data compilation of fruit consumption by representatives of the family Pteropodidae throughout their distributional range. To do so we performed an exhaustive search in Web of Science (http://apps.webofknowledge.com) with the following keywords and combinations: "flying foxes", "Pteropodidae”, “old World fruit bat”, "Megachiroptera", “Yinpterochiroptera”, "Chiroptera", "bat", "diet", "fruit", "frugivory", “frugivorous”, “dispersal”, “seed” and "plant”. We compiled data on species, genus and/or fruit family consumed, species and/or genus of consumer and geographical area. Each entry of a plant species (or genus/family when species unknown) accounted for one record.

We have found 220 studies and 2,415 records of fruit consumption by 33 bat genera out of 44 genera of Pteropodidae. Altogether, 601 species of fruit belonging to 325 genera from 106 botanical families were reported and this information will be presented elsewhere (Parolin et al in prep.). Here we have selected the three bat genera with the largest number of consumption records and broadest geographic distribution to compare them with the Neotropical genera (*Artibeus*, *Carollia* and *Sturnira*). We compiled the data in order to identify similar interactions to those observed in the Neotropics, in particular fruit choices or alleged preferences in the diet of these animals. Finally, we performed a search on comparative characteristics between the New and Old World bats including species richness, morphological characteristics, number of fruit species consumed, diet characteristics and IUCN Red List category to help explain the choices of bat genera.

We used Quantum GIS (QUANTUM GIS DEVELOPMENT TEAM, 2015) to build the maps of the overlap of the selected genera with the major zoographic regions (Holt et al. 2013). The distribution of bat species was obtained from the IUCN Red List (IUCN, 2014).

RESULTS

The three genera of Pteropodidae with the largest number of records of fruit consumption were *Pteropus* Brisson, 1762 with 33 bat species and 1,273 records (88 plant families and 231 fruit genera); *Cynopterus* F. Cuvier, 1824 with four species and 355 records (46 plant families and 95 fruit genera) and *Rousettus* with five species and 241 records (35 plant families and 66 fruit genera). Summed up these three genera represented more than 77% (N = 1869) out of consumption records of all Pteropodidae, including 99 plant families, 283 genera and more than 500 identified species in their diet.

Plant families frequently consumed by these three bats were: Moraceae (n = 453 records), Myrtaceae (n = 188), Sapotaceae (n = 102), Anacardiaceae (n = 101), Arecaceae (n = 62), Musaceae (n = 59), Rosaceae (n = 58), Combretaceae (n = 57), Annonaceae (n = 51), Caricaceae (n = 47), Leguminosae (n = 44), Rubiaceae (n = 42), Ebenaceae (n = 39), Clusiaceae (n = 37), Sapindaceae (n = 37), Meliaceae e Pandanaceae (n = 36), Rutaceae (n = 29), Elaeocarpaceae (n = 28) e Solanaceae (n = 21) (Table 1). The most consumed fruit genus, *Ficus* (Moraceae) represented 24.48% of all records of fruit consumption by *Rousettus,* 23.38% of all *Cynopterus* records and 17.91% of *Pteropus*.

Table 1. Families and genera of fruit with the highest number of consumption records for *Cynopterus*, *Pteropus* and *Rousettus*. The 20 most consumed genera per bat genera are mixed together, with the families in alphabetical order. Empty cells represent genera without data of fruit consumption by the bat genus.

Fruit	Bat genera		
	Cynopterus	***Pteropus***	***Rousettus***
Anacardiaceae	**15**	**76**	**10**
Mangifera L.	13	51	7
Annonaceae	**19**	**25**	**7**
Annona L.	9	11	5
Polyalthia Blume	8	7	2
Arecaceae	**17**	**31**	**14**
Phoenix L.	3	4	10
Caricaceae	**1**	**42**	**4**
Carica L.	1	42	4
Clusiaceae	**6**	**30**	**1**
Calophyllum L.	6	30	1
Combretaceae	**7**	**49**	**1**
Terminalia L.	7	49	1
Ebenaceae	**5**	**27**	**7**
Diospyros L.	5	27	7
Elaeocarpaceae	**7**	**20**	**1**
Elaeocarpus L.	7	20	1
Ericaceae	-	**4**	**4**
Arbutus L.	-	-	4
Leguminosae	**9**	**25**	**10**
Ceratonia L.	-	-	8
Inocarpus L.R. Forst & G.Forst	-	15	-
Pithecellobium Mart.	4	-	1
Malvaceae	**3**	**15**	**2**
Ceiba Mill.	-	13	1
Meliaceae	**5**	**16**	**15**
Azadirachta A.Juss	4	1	2
Melia L.	1	8	10
Moraceae	**89**	**296**	**68**
Artocarpus L.R. Forst & G.Forst	3	42	1
Ficus L.	83	228	59
Morus L.	3	14	8
Muntigiaceae	**6**	**3**	**2**
Muntingia L.	6	3	2

Musaceae	**11**	**42**	**6**
Musa L.	11	42	6
Myrtaceae	**34**	**132**	**22**
Psidium L.	10	43	9
Syzygium Gaertn.	14	71	10
Pandanaceae	**-**	**36**	**-**
Pandanus Parkinson	-	30	-
Piperaceae	**6**	**1**	**-**
Piper L.	6	1	-
Rhamnaceae	**5**	**10**	**1**
Ziziphus Mill.	5	4	1
Rosaceae	**2**	**34**	**22**
Eriobotrya Lindl.	-	7	10
Malus Mill.	-	8	3
Prunus L.	1	15	6
Rutaceae	**4**	**20**	**5**
Citrus L.	-	17	5
Sapindaceae	**6**	**26**	**5**
Litchi Sonn.	1	2	3
Sapotaceae	**35**	**59**	**8**
Achras L.	9	1	4
Palaquium Blanco	7	5	-
Planchonella Pierre	-	25	-
Solanaceae	**8**	**10**	**3**
Solanum L.	8	6	2
Vitaceae	**5**	**9**	**3**
Vitis L.	5	4	3

These three flying foxes are distributed across different zoogeographic regions in Africa, Asia and Oceania (Figure 1). *Cynopterus* is present only in the Oriental realm, *Pteropus* species are found in the Afrotropical, Australian, Madagascan, Oceanian, Oriental, Saharo-Arabian and Sino-Japanese and *Rousettus* is distributed in the Afrotropical, Madagascan, Oceanian, Oriental, Paleartic and Saharo-Arabian realms (Figure 1).

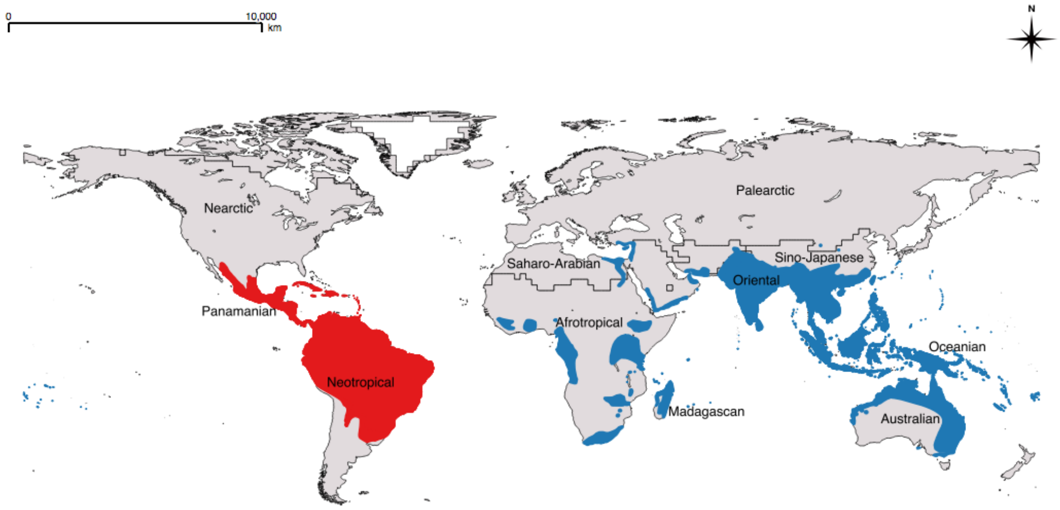

Figure 1. World map of Zoogeographic realms (Holt et al. 2013), with the distribution of *Artibeus*, *Carollia* and *Sturnira* in red and *Cynopterus*, *Pteropus* and *Rousettus* in blue (IUCN, 2014).

Among the Phyllostomidae there are only one endangered species, *Sturnira nana* Gardner and O'Neill, 1971, while among the Pteropodidae, 38 species are listed in some threatened category (CR, EN or VU) or went extinct (Table 2).

Table 2. Number and status for each bat genera based on IUCN (2016).

	Phyllostomidae (N = 33 species)			Pteropodidae (N = 83)		
	Artibeus	***Carollia***	***Sturnira***	***Cynopterus***	***Pteropus***	***Rousettus***
Category/ number of species	LC (n=9); DD (n=2)	LC (n=6)	EN (n=1); NT(n=1); LC(n=10); DD (n=4)	LC (n=8)	EX (n=4); CR (n=5) EN (=7); VU(n=19); NT (n=9); LC(n=12); DD (n=9)	VU (n=3); NT (n=1); LC (n=5); DD (n=1)

Categories: EX – Extinct; CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT – Near threatened; LC – Least Concern and DD – Data Deficient.

When we analyzed the spatial consistency of fruit choice among the three flying foxes in each zoogeographic realms, *Ficus* predominated in all combinations of region-bat except one (Table 3). And when we analyzed this consistency among the species of flying foxes, we also found that: 1. all species of *Cynopterus* and *Rousettus* exhibited higher consumption of fig species compared to other fruit, 2. among the 33 species of *Pteropus* with data on diet, 20 consumed more *Ficus* than any other species and other 12 also consumed *Ficus* but revealed no preference for this fruit; only one species, *Pteropus pselaphon* Lay, 1829 had no record of fig consumption so far.

Table 3. Predominant (based on the number of records) genera of fruit in the diet of three Pteropodidae within different zoogeographic regions. Empty cells represent realms where that bat genus does not occur.

Zoogeographic realms	**Bat genera**		
	Cynopterus	***Pteropus***	***Rousettus***
Afrotropical	-	*Ficus*	*Ficus*
Australian	-	*Ficus*	-
Madagascan	-	*Ficus*	*Ficus*
Oceanian	-	*Ficus*	*Ficus*
Oriental	*Ficus*	*Ficus*	*Ficus*
Paleartic	-	-	*Prunus*
Saharo-Arabian	-	*Ficus*	*Ficus*
Sino-Japanese	-	*Ficus*	-

So, when the three selected genera of pteropodids were compared with the three phyllostomids (Table 4), we observe that even though the diet of the later is less diverse, the preferred genera of fruit varied with the genera of bat.

Table 4. Number of species and frugivorous diet parameters for three Phyllostomidae and Pteropodidae genera. Superscript numbers indicate the source of the information.

Characteristics	Phyllostomidae (N = 33)			Pteropodidae (N = 83)		
	Artibeus	***Carollia***	***Sturnira***	***Cynopterus***	***Pteropus***	***Rousettus***
Number of species[1,2]	11	6	16	8	65	10
Number of species with diet records	11[3]	5[3]	6[3]	4[4]	33[4]	5[4]
Number of fruit genera in the diet	135[3]	78[3]	54[3]	95[4]	231[4]	66[4]
Genus most consumed	*Ficus*[3]	*Piper*[3]	*Solanum*[3]	*Ficus*[4]	*Ficus*[4]	*Ficus*[4]

Sources: 1.Simmons (2005); 2.IUCN (2016); 3.Parolin et al. (2016); 4.This paper.

DISCUSSION

Cynopterus, *Pteropus* and *Rousettus,* three out of 42 genera of Pteropodidae, are expected to be the Old World potential equivalents for the role played by three genera of Phyllostomidae in forest restoration. This assumption is based on their wide geographic distribution and diet composed by hundreds of fruit species, most of them known or expected to be dispersed by these bats. Besides, these three genera of flying foxes revealed a widespread (among species and regions) preference for fruits of *Ficus* spp*.,* whose essential oils already proved to attract seed dispersing phyllostomids to degraded land.

The genus *Pteropus* has 65 described species widely distributed, mainly on oceanic islands (SIMMONS, 2005). Such richness encompasses a huge variation in size, with forearms varying from 85 to 228 mm and wingspans of 610 to 1,700 mm (NOWAK, 1999), including one of the biggest bats in the World, *P. vampyrus* Linnaeus, 1758 (KUNZ; JONES, 2000). Although they are known to be able to fly up to 300 km (based on their presence on several Pacific islands), they usually travel 30 - 60 km to reach feeding areas (LIM, 1966; NOWAK, 1999; RIDLEY, 1930). As expected by the large number of species within this bat genus, the diet of *Pteropus* is the richest among pteropodids, with 231 fruit genera consumed, so that they should have huge impact on seed dispersal of hundreds of species.

Cynopterus has eight species distributed throughout the Eastern region of the Old World (SIMMONS, 2005). With a forearm varying between 55 and 92 mm and 305 to 457 mm wingspan (NOWAK, 1999). Despite of their smaller size compared to *Pteropus and Rou*settu*s,* and consequent lower linear distance of flight, they can travel more than 110 km per night (NOWAK, 1999). Besides, they have the ability to maneuver in partially enclosed sub-forest environments (CAMPBELL; KUNZ, 2006; MCKENZIE et al., 1995), which is important, from the restoration perspective, to give them access to plant species only found in this habitats. So it is not surprising that in spite of being more restricted geographically and less diverse, they were found to consume 95 different fruit genera, loosing only to *Pteropus*, whose distribution is not only wider but it also contains eight times more species.

Rousettus, a genus with 10 species distributed in several areas in the Old World and Oceania, is the only one present in the Paleartic realms. It has a forearm between 65 and 103 mm, a wingspan of up to 600 mm (KWIECINSKI; GRIFFITHS, 1999; NOWAK, 1999) and flight distances ranging between 25 and 60 km (MARSHALL, 1983; RIDLEY, 1930). Even though its diet is the least diverse among the three studied bats, it contains at least 66 fruit genera, which is a considerable number in restoration terms. Unlike other flying foxes, *Rousettus* echolocate, but they use the tongue to produce the echoes, differing from microbats that produce them through laryngeal vocalizations (HOLLAND; WATERS; RAYNER, 2004; YOVEL; GEVA-SAGIV; ULANOVSKY, 2011).

The sum of the genera of fruits consumed by these three Old World bats is approximately 1.6 times higher than those consumed by their three Neotropical counterparts (N = 176 see Parolin et al. 2016) and they also have a broader diet than phyllostomids in general (DUMONT, 2003). Fleming (1993) and Banack (1998) argue this difference is due to the environment conditions where pteropodids occur with irregular distributions, greater limitations in the availability of resources, and regions subject to periodic disturbance, promoting a more generalized feeding strategy.

Despite this generalistic profile, the diet of flying foxes had the highest number of records for consumption of Moraceae, specifically the genus *Ficus*. This fruit is widely consumed by several species of pteropodids (Parolin et al. in prep.) and is widely distributed throughout the globe (SHANAHAN et al., 2001). Due to the observed consistency of consumption throughout the distribution of these three genera of bats, it is possible to infer that these bats have a preference for fig species, in multiple localities and among many species.

Pteropodids are also considered to be an economic problem for orchards, generating a financial and environmental conflict, leading to the eradication of populations close to plantations (MICKLEBURGH; HUTSON; RACEY, 1992; SINGARAVELAN; MARIMUTHU, 2006). Despite this, several fruit species of commercial importance depend on seed dispersion by bats, and they can assist in mitigating the genetic erosion of these species (FUJITA; TUTTLE, 1991). Several genera of economic importance were found to have high consumption rates, such as *Carica* (papaya), *Ceiba* (kapok), *Citrus* (orange), *Eriobotrya* (loquat), *Litchi* (litchi), *Mangifera* (mango), *Musa* (plantain), *Prunus* (plum, peach, cherry), *Psidium* (guava) and *Syzygium* (rose apple), all widely cultivated fruits of economic importance (FUJITA; TUTTLE, 1991; WILES; FUJITA, 1992). Some authors argue that these bats do not always represent problems for these plantations but when they do, it is due to low availability of natural resources in their habitats (ANDRIANAIVOARIVELO et al., 2013; KORINE; IZHAKI; ARAD, 1999).

The processing of fruit by Pteropodidae consists of squeezing the entire pulp and spitting the remains of fibers and seeds near or a few meters away from the parent plant (BANACK, 1998; DUMONT, 2003). Although in the process they swallow some seeds, it is believed that this form of feeding is less efficient in the dispersion of seeds, since fast-eaters like *Carollia* and *Sturnira* chew the fruits and swallow a good part of the seeds, facilitating transport to more distant areas (DUMONT, 2003). Muscarella and Fleming (2007) even mention that phyllostomids spread a greater number of pioneer plants, mainly due to the profile of fruits that they most consume, smaller and with smaller seeds, than pteropodids. However, as can be seen from the diet data presented in this study, flying fox genera can also disperse species known to be important in the early stages of succession, such as *Ceiba*, *Musa*, *Solanum* and *Terminalia*. In addition, the dispersion of seeds such as those of the genus *Ficus*, well represented in their diet, may serve to generate recruitment foci, attracting other potential dispersers when fully grown (MUSCARELLA; FLEMING, 2007). The genera of Pteropodidae are also able to fly greater distances, of great importance in the arrival of propagules, in clearings and in more distant places, like the islands of the Pacific (DOCTERS VAN LEEUWEN, 1935; FUJITA; TUTTLE, 1991; THORNTON et al., 2001; THORNTON; COMPTON; WILSON, 1996).

The distribution of the three pteropodids is patchy throughout the countries and biomes of the Old World and Oceania. Much of this distribution, mainly in the Pacific islands, is commonly affected by tropical storms and typhoons, which can wipe out much of the local flora and fauna (ESSELSTYN; AMAR; JANEKE, 2006; MICKLEBURGH; HUTSON; RACEY, 2002). These locations vary in percentage of forested area, with places with large portions as well as areas with less than 5% remaining forest (HOEKSTRA et al., 2010). Many of their species are in some risk of threat, the genus *Pteropus* has a high number of endangered species, with four already extinct and more than 30 considered in some way threatened. The causes are related to loss of habitat, extermination to protect plantations, and control of diseases (MICKLEBURGH; HUTSON; RACEY, 2002; MICKLEBURGH; WAYLEN; RACEY, 2009). Phyllostomids, on the other hand are concentrated in South, Central, and part of North America, where vegetation cover ranges between 5 and 75%, with the exception of Amazonia, with more than 75% (HOEKSTRA et al., 2010). The number of threatened species is lower than the former, with only three endangered. But the fragmentation and habitat reduction for the all the six frugivorous in question is driven by the expansion of agriculture, timber extraction, and the development and extension of human infrastructure (GEIST; LAMBIN, 2002; SOUTHGATE, 1990).

Olfaction is an important aspect to mentioned, especially as it relates to a tool that uses essential oils to attract dispersers (BIANCONI et al., 2007; MIKICH et al., 2003). Ratcliffe (1932) observed species of *Pteropus* in Australia, and commented: "In their search for food, the bats are undoubtedly guided principally by their sense of smell". Fruit bats in general are recognized by this olfactory ability (ACHARYA et al., 1998; FLEMING, 1988; HAYDEN et al., 2014; LASKA, 1990; RIEGER; JAKOB, 1988; THIES; KALKO; SCHNITZLER, 1998) and *Cynopterus*, *Pteropus* and *Rousettus* are not different (e.g. Luft et al. 2003, Hodgkison et al. 2007). Over the years, phylogenetic analyses (HAYDEN et al., 2014), free-living observations (e.g. Ratcliffe 1932, van der Pijl 1957), and captive experiments have studied their choice against mimetic fruits with or without natural odor (ACHARYA et al., 1998; ELANGOVAN; PRIYA; MARIMUTHU, 2006; GANESH et al., 2010; HODGKISON et al., 2003, 2007, 2013; LUFT; CURIO; TACUD, 2003; RAGHURAM et al., 2009; TANG et al., 2007; ZHANG et al., 2014) and with different chemical compounds important in fruit maturation (SÁNCHEZ et al., 2004, 2006), which confirm the importance of smell in the selection of mature fruits. In a recent study, Hodgkison et al. (2013) in captive experiments, offered the raw essential oil of different *Ficus* fruits for *Artibeus* and *Cynopterus*, obtaining more positive responses for the former; *Cynopterus* only showed interest towards the smell of the Old World figs. This information could help discover which are the compounds that serve as the specific attractants.

In conclusion, the key to the implementation of the technique - which attracts frugivorous bats to degraded areas with the aim of increasing seed rain and favoring natural regeneration - is a combination of necessary factors. Species must be good dispersers with a diverse diet showing some level of preference or greater consumption to a particular food resource, that can be used as an olfactory decoy (BIANCONI et al., 2007, 2012; MIKICH et al., 2003). All these characteristics are presented by flying foxes *Cynopterus*, *Pteropus* and *Rousettus*. Combining all these characteristics enable us to draw parallels for the Neotropical conservation tool between the New World phyllostomids and flying foxes and the possibility of trial implementation of this approach in several degraded sites of the Old World.

Experiments in the wild and captivity must be carried out to observe the behavioral response of flying foxes to the essential oil of *Ficus*. The key is to determine if they present the same behavioral response as the Neotropical bats - perceive, identify and spend time close to the stimulus - in order to efficiently increase seed rain. In addition, as is already being analyzed in Brazil for phyllostomids (Parolin et al. in prep.), it is necessary to uncover which compound or compound combination is in fact responsible for the attraction of fruit bats. This will allow the synthesis of such compound(s) and its widespread use in restoration initiatives. Perhaps as important, this type of activity can generate positive responses regarding bats among the general public, who will see these animals not only as crop destroyers or disease transmitters, but also as species that can provide an important ecological service at little or no cost at all.

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CONSIDERAÇÕES FINAIS

O conjunto de resultados obtidos fornece subsídios claros para a implantação da técnica de recuperação de ecossistemas florestais degradados baseada na atração de morcegos dispersores na Região Neotropical e para a sugestão de sua expansão para as demais regiões tropicais do Mundo. Além de elucidar questões sobre a escolha química de morcegos frugívoros e inserir novas possibilidades de estudos na ecologia química, esta tese abre portas para pesquisadores que queiram entender e testar estes processos com outros grupos animais e mesmo inserir a técnica em diferentes locais do Globo.

No Capítulo I há a confirmação de semelhanças entre óleos essenciais (compostos orgânicos voláteis) de frutos preferidos pelos filostomídeos frugívoros *Artibeus*, *Carollia* e *Sturnira* com os óleos encontrados em outros frutos que consomem. Além disso, os dados ressaltam a possibilidade de que alguns compostos-chave possam caracterizar os frutos preferidos por estes morcegos.

O Capítulo II aprofunda o tema dos compostos orgânicos voláteis, evidenciando que morcegos frugívoros conseguem identificar dois tipos de compostos. Estes teriam diferentes funções: os monoterpenos atuariam como sinais breves e importantes para a identificação e localização do recurso alimentar; já os sesquiterpenos atuariam como um sinal mais duradouro para indicar a qualidade (grau de maturação) do mesmo.

Os capítulos III e IV abordam uma família de morcegos do Velho Mundo, Pteropodidae, analisando a sua dieta e, com base nela, a possibilidade de uso da mesma ferramenta de restauração de áreas degradadas desenvolvida com base na dieta e comportamento de morcegos da família Phyllostomidae, encontrada na região Neotropical. Verificou-se que os pteropodídeos possuem uma dieta frugívora bastante diversa, mas praticamente todos os gêneros exibem preferência por frutos do gênero *Ficus,* independente da região em que vivem (Capítulo III). Por fim, o Capítulo IV traz os gêneros *Cynopterus*, *Pteropus* e *Rousettus* como modelos para o Velho Mundo, assim como o são *Artibeus*, *Carollia* e *Sturnira* para a técnica de restauração nas Américas. Além de vasta distribuição, dieta diversa e preferência por frutos de *Ficus*, possuem diversas espécies em risco de extinção, de modo que seu uso em programas de restauração ambiental poderia ainda favorecer a imagem destes animais, contribuindo com sua conservação.

Appendix S1 - Bat species or genera and the number of fruit consumption records

Bat species/genera	**Nrec**
Acerodon jubatus Eschscholtz, 1831	9
Acerodon mackloti Temminck, 1837	1
Aproteles bulmerae Menzies, 1977	2
Balionycteris maculata Thomas, 1893	24
Chironax melanocephalus Temminck, 1825	9
Cynopterus brachyotis Müller, 1838	134
Cynopterus F. Cuvier, 1824.	6
Cynopterus horsfieldii Gray, 1843	20
Cynopterus sphinx Vahl, 1797	188
Cynopterus titthaecheilus Temminck, 1825	7
Desmalopex microleucopterus Esselstyn, Garcia, Saulog & Heaney, 2008	3
Dobsonia anderseni Thomas, 1914	3
Dobsonia chapmani Rabor, 1952	1
Dobsonia magna Thomas, 1905	1
Dobsonia minor Dobson, 1878	2
Dobsonia moluccensis Quoy and Gaimard, 1830	12
Dobsonia peroni E. Geoffroy, 1810	3
Dobsonia praedatrix K. Andersen, 1909	1
Dyacopterus spadiceus Thomas, 1890	6
Eidolon dupreanum Pollen In Schlegel and Pollen, 1866	29
Eidolon helvum Kerr, 1792	113
Eidolon Rafinesque, 1815	1
Eonycteris spelaea Dobson, 1871	1
Epomophoros labiatus Temminck, 1837	1
Epomophorus angolensis Gray, 1870	3
Epomophorus Bennett, 1835	16
Epomophorus crypturus Peters, 1852	1
Epomophorus gambianus Ogilby, 1835	43
Epomophorus labiatus Temminck, 1837	3
Epomophorus minor Dobson, 1879	2
Epomophorus wahlbergi Sundevall, 1846	64
Epomops buettikoferi Matschie, 1899	21
Epomops franqueti Tomes, 1860	13
Epomops Gray, 1870	3
Haplonycteris fischeri Lawrence, 1939	2
Harpyionycteris whiteheadi Thomas, 1896	2
Hypsignathus monstrosus H. Allen, 1861	24
Latidens salimalii Thonglongya, 1972	11
Lissonycteris angolensis Bocage, 1898	12
Macroglossus F. Cuvier, 1824	1
Megaerops ecaudatus Temminck, 1837	6
Megaerops wetmorei Taylor, 1934	1
Melonycteris (Melonycteris) melanops Dobson, 1877	2
Micropteropus pusillus Peters, 1867	21

Appendix S1 - Continuation

Bat species/genera	**Nrec**
Myonycteris (Myonycteris) torquata Dobson, 1878	7
Nanonycteris Matschie, 1899	1
Nanonycteris veldkampii Jentink, 1888	3
Nyctimene aello Thomas, 1900	1
Nyctimene major Dobson, 1877	2
Nyctimene robinsoni Thomas, 1904	10
Nyctimene vizcaccia Thomas, 1914	1
Otopteropus cartilagonodus Kock, 1969	1
Paranyctimene raptor Tate, 1942	2
Ptenochirus jagori Peters, 1861	16
Ptenochirus minor Yoshiyuki, 1979	1
Ptenochirus Peters, 1861	2
Pteralopex Thomas, 1888	16
Pteropus aldabrensis True, 1893	5
Pteropus alecto Temminck, 1837	58
Pteropus anetianus Gray, 1870	3
Pteropus banakrisi Richards and Hall, 2002	2
Pteropus conspicillatus Gould, 1849	88
Pteropus dasymallus Temminck, 1825	167
Pteropus giganteus Brünnich, 1782	110
Pteropus griseus E. Geoffroy, 1810	3
Pteropus hypomelanus Temminck, 1853	10
Pteropus leucopterus Temminck, 1853	2
Pteropus livingstonii Gray, 1866	3
Pteropus loochoensis Gray, 1870	4
Pteropus macrotis Peters, 1867	1
Pteropus mariannus Desmarest, 1822	98
Pteropus melanotus Blyth, 1863	24
Pteropus molossinus Temminck, 1853	2
Pteropus neohibernicus Peters, 1876	6
Pteropus niger Kerr, 1792	32
Pteropus ornatus Gray, 1870	8
Pteropus pelagicus Kittlitz, 1836	7
Pteropus pelewensis K. Andersen, 1908	64
Pteropus poliocephalus Temminck, 1825	151
Pteropus pselaphon Lay, 1829	12
Pteropus pumilus Miller, 1911	2
Pteropus rodricensis Dobson, 1878	9
Pteropus rufus E. Geoffroy, 1803	71
Pteropus samoensis Peale, 1848	87
Pteropus scapulatus Peters, 1862	23
Pteropus seychellensis Milne-Edwards, 1877	47
Pteropus subniger Kerr, 1792	2
Pteropus tokudae Tate, 1934	4
Pteropus tonganus Quoy and Gaimard, 1830	125

Appendix S1 - Continuation

Bat species/genera	**Nrec**
Pteropus vampyrus Linnaeus, 1758	29
Pteropus voeltzkowi Matschie, 1909	14
Rousettus (Rousettus) aegyptiacus E. Geoffroy, 1810	139
Rousettus (Rousettus) amplexicaudatus E. Geoffroy, 1810	12
Rousettus (Rousettus) leschenaultii Desmarest, 1820	69
Rousettus (Rousettus) obliviosus Kock, 1978	10
Rousettus (Stenonycteris) madagascariensis G. Grandidier, 1928	11
Scotonycteris ophiodon Pohle, 1943	1
Scotonycteris zenkeri Matschie, 1894	2
Syconycteris australis Peters, 1867	6
Thoopterus nigrescens Gray, 1870	1

Nrec= number of records

Appendix S2 - Plant coordinates from Non-Metric Multidimensional Scaling (NMDS) of realms by fruit present on frugivorous Pteropodidae diet

Plant genus or family	**Axis 1**	**Axis 2**
Alpinia L.	-1.27064	0.63865
Ampelopsis Michx.	-1.27064	0.63865
Ardisia Gaertn.	-1.27064	0.63865
Eurya Thunb.	-1.27064	0.63865
Ginkgo L.	-1.27064	0.63865
Pieris D.Don	-1.27064	0.63865
Arbutus L.	-0.6557	0.89543
Ceratonia L.	-0.6557	0.89543
Crataegus L.	-0.6557	0.89543
Lantana L.	-0.6557	0.89543
Styrax L.	-0.6557	0.89543
Washingtonia H. Wendl.	-0.6557	0.89543
Elaeagnus L.	-0.55523	-0.15107
Rhamnus L.	-0.55523	-0.15107
Actinidia Lindl.	-0.41215	-0.30901
Areca L.	-0.24776	-0.02268
Morus L.	-0.23967	-0.02462
Garcinia L.	-0.19752	-0.54593
Melia L.	-0.16836	-0.06112
Cocos L.	-0.16469	-0.33483
Vaccinium L.	-0.11804	0.27499
Phoenix L.	-0.11277	0.0954
Bischofia Blume	-0.07829	-0.67755
Punica L.	-0.05036	-0.56503
Citrus L.	0.00704	-0.10411
Prunus L.	0.05408	-0.32897
Eriobotrya Lindl.	0.09395	-0.1764
Vitis L.	0.09681	-0.58413
Nephelium L.	0.10675	-0.76302
Malus Mill.	0.11071	-0.10048
Diospyros L.	0.13065	-0.43583
Polyalthia Blume	0.14617	-0.71779
Elaeocarpus L.	0.1592	-0.68569
Acacia Mill.	0.16018	-0.94079
Achras L.	0.16018	-0.94079
Adinandra Jack	0.16018	-0.94079
Agave L.	0.16018	-0.94079
Ananas Mill.	0.16018	-0.94079
Anthocephalus A.Rich.	0.16018	-0.94079
Attalea Kunth	0.16018	-0.94079
Bassia All.	0.16018	-0.94079
Broussonetia L'Hér. ex Vent.	0.16018	-0.94079
Calamus L.	0.16018	-0.94079
Caryota L.	0.16018	-0.94079
Cassia L.	0.16018	-0.94079
Cephalandra Schrad. ex Eckl. & Zeyh.	0.16018	-0.94079

Appendix S2 - Continuation

Plant genus or family	**Axis 1**	**Axis 2**
Chrysalidocarpus H.Wendl.	0.16018	-0.94079
Chrysophyllum L.	0.16018	-0.94079
Coccinia Wight & Arn.	0.16018	-0.94079
Cyathocalyx Champ. ex Hook.f. & Thomson	0.16018	-0.94079
Cyrtophyllum Reinw. ex Blume	0.16018	-0.94079
Dillenia L.	0.16018	-0.94079
Dimocarpus Lour.	0.16018	-0.94079
Diplospora DC.	0.16018	-0.94079
Durio Adans.	0.16018	-0.94079
Elaeis Jacq.	0.16018	-0.94079
Enterolobium Mart.	0.16018	-0.94079
Gnetum L.	0.16018	-0.94079
Gonystylus Teijsm. & Binn.	0.16018	-0.94079
Guazuma Mill.	0.16018	-0.94079
Idesia Maxim.	0.16018	-0.94079
Lannea A. Rich. in Guillem.	0.16018	-0.94079
Lansium Corrêa	0.16018	-0.94079
Lauraceae Juss.	0.16018	-0.94079
Licuala Wurmb	0.16018	-0.94079
Macaranga Thouars	0.16018	-0.94079
Madhuca J.F.Gmel.	0.16018	-0.94079
Malpighia Plum. ex L.	0.16018	-0.94079
Melanolepis Rchb. & Zoll.	0.16018	-0.94079
Melastomataceae Juss.	0.16018	-0.94079
Memecylon L.	0.16018	-0.94079
Mezzettia Becc.	0.16018	-0.94079
Microcos Burm. ex L.	0.16018	-0.94079
Murraya J.Koenig	0.16018	-0.94079
Myrica L.	0.16018	-0.94079
Octomeles Miq.	0.16018	-0.94079
Osmanthus Lour.	0.16018	-0.94079
Oxalidaceae R.Br.	0.16018	-0.94079
Payena A. DC.	0.16018	-0.94079
Pellacalyx Korth.	0.16018	-0.94079
Pinanga Blume	0.16018	-0.94079
Plectocomiopsis Becc.	0.16018	-0.94079
Prosopis L.	0.16018	-0.94079
Pseuduvaria Miq.	0.16018	-0.94079
Pternandra Jack	0.16018	-0.94079
Ptychosperma Labill.	0.16018	-0.94079
Putranjiva Wall.	0.16018	-0.94079
Pygeum Gaertn.	0.16018	-0.94079
Rhodomyrtus (DC.) Rchb.	0.16018	-0.94079
Roystonea O.F.Cook	0.16018	-0.94079
Santalum L.	0.16018	-0.94079
Sapota Mill.	0.16018	-0.94079

Appendix S2 - Continuation

Plant genus or family	Axis 1	Axis 2
Saurauia Willd.	0.16018	-0.94079
Shorea Roxb. ex C.F.Gaertn.	0.16018	-0.94079
Stephania Lour.	0.16018	-0.94079
Strombosia Blume	0.16018	-0.94079
Strychnos L.	0.16018	-0.94079
Trema Lour.	0.16018	-0.94079
Turpinia Vent.	0.16018	-0.94079
Xerospermum Blume	0.16018	-0.94079
Muntingia L.	0.17919	-0.93085
Pithecellobium Mart.	0.1982	-0.92092
Ficus L.	0.20461	-0.49511
Cyrtandra J.R.Forst. & G.Forst.	0.2058	-0.91695
Ziziphus Mill.	0.21374	-0.67841
Eugenia L.	0.22053	-0.69177
Palaquium Blanco	0.22101	-0.909
Chionanthus D.Royen	0.23621	-0.90105
Nauclea L.	0.23957	-0.70023
Livistona R.Br.	0.24398	-0.60324
Schefflera J.R.Forst. & G.Forst.	0.24398	-0.60324
Manilkara Adans.	0.25154	-0.67272
Timonius DC.	0.25406	-0.69588
Pouteria Aubl.	0.25794	-0.8897
Piper L.	0.25836	-0.83606
Melastoma L.	0.27423	-0.88118
Sapotaceae Juss.	0.27423	-0.88118
Calophyllum L.	0.28305	-0.84565
Canarium L.	0.28494	-0.75808
Pyrus L.	0.28588	-0.43446
Musa L.	0.2912	-0.65957
Pandanus Parkinson	0.29182	-0.78649
Pipturus Wedd.	0.29704	-0.86926
Fagraea Thunb.	0.30274	-0.86628
Annona L.	0.31263	-0.74609
Psidium L.	0.31922	-0.54507
Freycinetia Gaudich.	0.32311	-0.85563
Syzygium Gaertn.	0.32729	-0.63464
Achronychia J.R.Forst. & G.Forst.	0.32778	-0.26568
Acmena DC.	0.32778	-0.26568
Aidia Lour.	0.32778	-0.26568
Angophora Cav.	0.32778	-0.26568
Archontophoenix H.Wendl. & Drude	0.32778	-0.26568
Arecaceae Bercht. & J.Presl	0.32778	-0.26568
Arecastrum (Drude) Becc.	0.32778	-0.26568
Buchanania Spreng.	0.32778	-0.26568
Carpentaria Becc.	0.32778	-0.26568
Cissus L.	0.32778	-0.26568

Appendix S2 - Continuation

Plant genus or family	**Axis 1**	**Axis 2**
Cudrania Trécul	0.32778	-0.26568
Davidsonia F.Muell.	0.32778	-0.26568
Decaspermum J.R.Forst. & G.Forst.	0.32778	-0.26568
Diploglottis Hook.f.	0.32778	-0.26568
Drypetes Vahl	0.32778	-0.26568
Ehretia P.Browne	0.32778	-0.26568
Hedycarya J.R.Forst. & G.Forst.	0.32778	-0.26568
Laurus L.	0.32778	-0.26568
Ligustrum L.	0.32778	-0.26568
Maclura Nutt.	0.32778	-0.26568
Mallotus Lour.	0.32778	-0.26568
Melaleuca L.	0.32778	-0.26568
Maranthes Blume	0.32778	-0.26568
Myristica Gronov.	0.32778	-0.26568
Notothixos Oliv.	0.32778	-0.26568
Oreodoxa Willd.	0.32778	-0.26568
Pennantia J.R.Forst. & G.Forst.	0.32778	-0.26568
Pittosporum Banks ex Gaertn.	0.32778	-0.26568
Polyosma Blume	0.32778	-0.26568
Randia L.	0.32778	-0.26568
Rauwenhoffia Scheff.	0.32778	-0.26568
Rhodamnia Jack	0.32778	-0.26568
Rollinia A.St.-Hil.	0.32778	-0.26568
Schinus L.	0.32778	-0.26568
Schizomeria D.Don	0.32778	-0.26568
Syagrus Mart.	0.32778	-0.26568
Terminalia L.	0.32907	-0.6754
Cycas L.	0.33125	-0.85137
Rhus L.	0.33125	-0.85137
Sapium P.Browne	0.34102	-0.72736
Mangifera L.	0.34176	-0.63694
Tristiropsis Radlk.	0.34266	-0.84541
Erythrina L.	0.34421	0.03355
Tamarindus L.	0.34581	-0.1741
Dendrocnide Miq.	0.34795	-0.45098
Melodinus J.R.Forst. & G.Forst.	0.34795	-0.45098
Anacardium L.	0.34841	-0.70252
Planchonella Pierre	0.34881	-0.7247
Mimusops L.	0.35391	-0.75305
Azadirachta A.Juss	0.35494	-0.71094
Borassus L.	0.35779	-0.65985
Alphitonia Reissek ex Endl.	0.35803	-0.54362
Cupaniopsis Radlk.	0.35803	-0.54362
Faradaya F.Muell.	0.35803	-0.54362
Gmelina L.	0.35803	-0.54362
Flacourtia Comm. ex L'Hér.	0.3589	-0.25149

Appendix S2 - Continuation

Plant genus or family	Axis 1	Axis 2
Inocarpus L.R. Forst & G.Forst	0.36904	-0.79246
Artocarpus L.R. Forst & G.Forst	0.3766	-0.76631
Carica L.	0.37665	-0.60791
Morinda L.	0.3803	-0.49235
Eucalyptus L'Her.	0.38466	-0.207
Aglaia Lour.	0.38828	-0.82157
Antidesma L.	0.38828	-0.82157
Barringtonia J.R.Forst. & G.Forst.	0.38828	-0.82157
Burckella Pierre	0.38828	-0.82157
Campnosperma Thwaites	0.38828	-0.82157
Cananga Aubl.	0.38828	-0.82157
Castanospermum A.Cunn. ex Mudie	0.38828	-0.82157
Castilla Cerv.	0.38828	-0.82157
Casuarina L.	0.38828	-0.82157
Cerbera L.	0.38828	-0.82157
Cestrum L.	0.38828	-0.82157
Clinostigma H.Wendl.	0.38828	-0.82157
Collospermum Skottsb.	0.38828	-0.82157
Dracontomelon Blume	0.38828	-0.82157
Dysoxylum Blume	0.38828	-0.82157
Euphoria Comm. ex Juss.	0.38828	-0.82157
Exorrhiza Becc.	0.38828	-0.82157
Finschia Warb. & C.T.White	0.38828	-0.82157
Glochidion J.R.Forst. & G.Forst.	0.38828	-0.82157
Grangeria Comm. ex Juss.	0.38828	-0.82157
Guettarda L.	0.38828	-0.82157
Hernandia L.	0.38828	-0.82157
Horsfieldia Willd.	0.38828	-0.82157
Hyophorbe Gaertn.	0.38828	-0.82157
Labourdonnaisia Bojer	0.38828	-0.82157
Lycopersicum Hill	0.38828	-0.82157
Mammea L.	0.38828	-0.82157
Merrilliodendron Kaneh.	0.38828	-0.82157
Moraceae Gaudich.	0.38828	-0.82157
Neisosperma Raf.	0.38828	-0.82157
Neuburgia Blume	0.38828	-0.82157
Northia Hook.f.	0.38828	-0.82157
Ochrosia Juss.	0.38828	-0.82157
Osmoxylon Miq.	0.38828	-0.82157
Pangium Reinw.	0.38828	-0.82157
Piperaceae Giseke	0.38828	-0.82157
Pleiogynium Engl.	0.38828	-0.82157
Pometia J.R.Forst. & G.Forst.	0.38828	-0.82157
Premna L.	0.38828	-0.82157
Protium Burm.f.	0.38828	-0.82157
Pyrostria Comm. ex Juss.	0.38828	-0.82157

Appendix S2 - Continuation

Plant genus or family	**Axis 1**	**Axis 2**
Salacia L.	0.38828	-0.82157
Sandoricum Cav.	0.38828	-0.82157
Semecarpus L.f.	0.38828	-0.82157
Sonneratia L.f.	0.38828	-0.82157
Symplocos Jacq.	0.38828	-0.82157
Warneckea Gilg	0.38828	-0.82157
Ceiba Mill.	0.38885	-0.36733
Litchi Sonn.	0.39049	-0.62807
Persea Mill.	0.39479	-0.73889
Sideroxylon L.	0.39657	-0.33053
Parkia R.Br.	0.40131	-0.65622
Passiflora L.	0.40543	-0.20256
Grewia L.	0.40555	0.35833
Celtis L.	0.40804	-0.34133
Spondias L.	0.415	-0.76004
Solanum L.	0.41507	-0.44749
Cordia L.	0.43304	-0.24019
Allophylus L.	0.43493	-0.21175
Aphloia (DC.) Benn.	0.43493	-0.21175
Crateva L.	0.43493	-0.21175
Cassine L.	0.45508	-0.66775
Rubiaceae Juss.	0.45825	0.09316
Solanaceae Juss.	0.45825	0.09316
Podocarpus Labill.	0.46012	-0.53378
Rubus L.	0.47812	0.6895
Parinari Aubl.	0.48091	-0.52135
Dypsis Noronha ex Thou.	0.49324	0.55053
Acokanthera G.Don	0.52187	-0.51393
Adenia Forssk.	0.52187	-0.51393
Antiaris Lesch.	0.52187	-0.51393
Balanites Delile	0.52187	-0.51393
Berchemia Neck. ex DC.	0.52187	-0.51393
Bombax L.	0.52187	-0.51393
Bridelia Willd.	0.52187	-0.51393
Butyrospermum Kotschy	0.52187	-0.51393
Cecropia Loefl.	0.52187	-0.51393
Chlorophora Gaudich.	0.52187	-0.51393
Cola Schott & Endl.	0.52187	-0.51393
Cordiae L.	0.52187	-0.51393
Curtisina Ridl.	0.52187	-0.51393
Cydonia Mill.	0.52187	-0.51393
Ekebergia Sparrm.	0.52187	-0.51393
Euclea L.	0.52187	-0.51393
Halleria L.	0.52187	-0.51393
Harpephyllum Bernh. ex Krauss	0.52187	-0.51393
Irvingia Hook.f.	0.52187	-0.51393

Appendix S2 - Continuation

Plant genus or family	**Axis 1**	**Axis 2**
Kigelia DC.	0.52187	-0.51393
Maesopsis Engl.	0.52187	-0.51393
Magnistipula Engl.	0.52187	-0.51393
Milicia Sim	0.52187	-0.51393
Momordica L.	0.52187	-0.51393
Musanga C.Sm. ex R.Br.	0.52187	-0.51393
Olea L.	0.52187	-0.51393
Olinia Thunb.	0.52187	-0.51393
Pseudolachnostylis Pax	0.52187	-0.51393
Pseudospondias Engl.	0.52187	-0.51393
Pycnanthus Warb.	0.52187	-0.51393
Rauvolfia L.	0.52187	-0.51393
Smeathmannia Sol. ex R.Br.	0.52187	-0.51393
Tabernaemontana L.	0.52187	-0.51393
Trichilia P.Browne	0.52187	-0.51393
Uvaria L.	0.52187	-0.51393
Viscum L.	0.52187	-0.51393
Vitex L.	0.52187	-0.51393
Voacanga Thouars	0.52187	-0.51393
Sclerocarya Hochst.	0.52314	-0.20957
Uapaca Baill.	0.52378	-0.05738
Adansonia L.	0.52399	-0.00666
Anthocleista Afzel.ex R.Br.	0.52441	0.0948
Bakerella Tiegh	0.52823	1.00789
Beilschmiedia Nees	0.52823	1.00789
Brexia Noronha ex Thouars	0.52823	1.00789
Canephora Juss.	0.52823	1.00789
Canthium Lam.	0.52823	1.00789
Cassinopsis Sond.	0.52823	1.00789
Cinnamosma Baill.	0.52823	1.00789
Cussonia Thunb.	0.52823	1.00789
Dracaena Vand.	0.52823	1.00789
Gambeya Pierre	0.52823	1.00789
Hylocereus (A.Berger) Britton & Rose	0.52823	1.00789
Ilex L.	0.52823	1.00789
Ixora L.	0.52823	1.00789
Ludia Comm. ex Juss.	0.52823	1.00789
Ocotea Aubl.	0.52823	1.00789
Ophiocolea H.Perrier	0.52823	1.00789
Pauridiantha Hook.f.	0.52823	1.00789
Polyscias J.R.Forst. & G.Forst.	0.52823	1.00789
Psychotria L.	0.52823	1.00789
Rothmannia Thunb.	0.52823	1.00789
Sarcolaena Thouars	0.52823	1.00789
Scolopia Schreb.	0.52823	1.00789
Tambourissa Sonn.	0.52823	1.00789

Appendix S2 - Continuation

Plant genus or family	**Axis 1**	**Axis 2**
Tricalysia A.Rich. ex DC.	0.52823	1.00789
Vepris Comm. ex A.Juss.	0.52823	1.00789