UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

COMPOSIÇÃO, ABUNDÂNCIA E RESPOSTAS A VARIÁVEIS AMBIENTAIS DA ASSEMBLEIA DE TIPULOIDEA (INSECTA: DIPTERA) EM MARISMAS DO ESTUÁRIO DA LAGOA DOS PATOS

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PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA NÍVEIS MESTRADO E DOUTORADO

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Composição, abundância e respostas a variáveis ambientais da assembleia de Tipuloidea (Insecta: Diptera) em marismas do estuário da Lagoa dos Patos

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DEDICATÓRIA

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"Aqueles que se sentem satisfeitos sentam-se e nada fazem. Os insatisfeitos são os únicos benfeitores do mundo."

Walter S. Landor

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RESUMO

Ecossistemas estuarinos apresentam características singulares em termos de ciclagem de nutrientes, salinidade, flora e fauna. Os animais que ocupam estes ambientes necessitam de grande plasticidade para tolerar as bruscas alterações ambientais; esta fauna é composta por uma mistura entre grupos de origem marinha (bivalves, crustáceos, poliquetas) e terrestre (aves, mamíferos, insetos). Embora tenham grande importância ecológica como detritívoros/succívoros, pouco se conhece quanto à ocupação, distribuição e ecologia dos insetos, incluindo Tipuloidea, neste ambiente. O objetivo dessa dissertação foi identificar as espécies de tipulídeos (morfologica e molecularmente), gerar um checklist e uma chave de identificação. E também avaliar a composição, sazonalidade e efeitos de variáveis abióticas sobre a abundância das espécies de Tipuloidea em marismas do estuário da Lagoa dos Patos, ao sul da região Neotropical. Três pontos foram amostrados ao longo do corpo estuarino, instalando-se duas armadilhas Malaise em cada ponto, uma em cada estrato de marisma: baixa e alta. As coletas foram realizadas quinzenalmente entre agosto/2015 e julho/2016, e o material coletado foi triado e identificado. Como resultados do primeiro capítulo foram coletados 5.248 tipulídeos identificados em quinze espécies e dezessete morfoespécies pertencentes a Limoniidae e Tipulidae stricto sensu. Os limites de distribuição de Symplecta cana (Walker, 1848) e duas espécies de Ormosia Rondani, 1856 foram expandidos. Adicionalmente, um complexo de espécies de Dicranomyia Stephens, 1829 também foi registrado. As relações filogenéticas entre as subfamílias de Tipuloidea corroborou Tipulidae stricto sensu como um grupo monofilético e 'Limoniidae' como um agrupamento

artificial. E no segundo capítulo uma curva de acúmulo de espécies revelou maior número de espécies no estuário superior seguido do estuário inferior e estuário médio. A classificação das espécies guanto à sua freguência de ocorrência revelou uma lacuna de ocorrência de espécies constantes no estuário médio, possivelmente devido ao estresse causado pelas elevadas flutuações de salinidade na área. Espécies de Dicranomyia, Gonomyia, Teucholabis e Zelandotipula foram acessórias apenas no estuário superior, enquanto Symplecta cana foi acessória apenas no baixo estuário, constatando a preferência de cada uma das espécies pelas respectivas condições físicas dos pontos do estuário. Uma CCA sugeriu maior abundância da maioria das espécies em temperaturas e umidades elevadas e velocidades de vento baixas. Symplecta pilipes teve clara contraposição e aumento de abundância em temperaturas mais baixas. Um diagrama de Whittaker junto a uma análise de PCoA revelaram uma alternância sazonal na composição das espécies de Tipuloidea, onde ficou evidente a maior equabilidade das espécies de Dicranomyia, Geranomyia, Rhipidia domestica e Symplecta cana (15-20%) durante o verão, enquanto no inverno houve dominância de Symplecta pilipes (80%). Assim, tal padrão sugere diferenças constantes e sazonais na estruturação da assembleia de tipulídeos, gerando modificações temporais em sua composição nas marismas e, por conseguinte, potencial influência em níveis tróficos superiores.

Palavras-chave: insetos de marisma; Tipuloidea; identificação molecular; diversidade e estrutura; sazonalidade; *Symplecta pilipes*.

ABSTRACT

Estuarine ecosystems present unique characteristics in terms of nutrient cycling, salinity, habitats, flora and fauna. The animals that occupy salt marshes require physiological plasticity to tolerate the common environmental changes of these areas; such fauna is composed of a mixture of marine (bivalves, crustaceans, polychaetes) and terrestrial (birds, mammals, insects) animals. Despite their ecological importance as detritivorous/succivorous, little is known on the occupation, distribution and ecology of insects, including Tipuloidea, in these environments. This study aimed to identify crane-flies species (morphologically and genetically), construct a checklist, identification key. Also, to evaluate the composition, seasonality and effect of abiotic factors on the abundance, diversity and structure of a Tipuloidea assemblage at Patos Lagoon salt marshes, located at the south of the Neotropical region. We sampled crane-flies from three points along the estuary by installing two Malaise traps at the low and high marsh strata of each point. Sampling was conducted each fifteen days between August/2015 and July/2016. Collected insects were identified using genitalia and wings morphology. As result of first chapter, 5.248 crane-flies were identified in fifteen species and seventeen morphospecies belonging to Limoniidae and Tipulidae stricto sensu. Distibution limits for Symplecta cana (Walker, 1848) and two species of Ormosia Rondani, 1856 were expanded. Additionally, a Dicranomyia Stephens, 1829 species complex was also recorded. Phylogenetic relations between the Tipuloidea subfamilies corroborated that Tipulidae stricto sensu is a monophyletic group, while 'Limoniidae' is an artificial grouping. On the second chapter, a species accumulation curve showed a higher number of species in the upper estuary, followed by the middle and lower estuaries. Frequency of

occurrence of species revealed a gap in the presence of constant species at the middle estuary, possibly due to stress caused by large fluctuations in salinity at the area. *Dicranomyia, Gonomyia, Teucholabis* and *Zelandotipula* species were accessory only at the upper estuary, while *Symplecta cana* was accessory only at the lower estuary. This shows that different species prefer distinct points along the estuary. A CCA analysis suggests a tendency for higher abundance of crane-flies under elevated temperature and humidity, and low winds. *Symplecta pilipes* was an exception, presenting increase in abundance under lower temperatures. A Whittaker diagram and a PCoA revealed seasonal change in Tipuloidea species composition, with higher evenness of *Dicranomyia, Geranomyia, Rhipidia domestica* and *Symplecta cana* (15-20%) during summer, and dominance of *Symplecta pilipes* in winter (80%). These seasonal differences can have significant ecological consequences such as modification of the Tipuloid species composition, and consequently influence the upper trophic levels of the salt marsh environment.

Keywords: salt marsh insects; Tipuloidea; DNA barcoding; diversity and structure; seasonality; *Symplecta pilipes*.

INTRODUÇÃO

Ecossistemas costeiros compõem vastas áreas ao redor do globo; apresentam características singulares em termos de ciclagem de nutrientes, variação climática, características geomorfológicas, composição de fauna e flora (LALLI & PARSONS 2006; REZENDE *et al.* 2009). Dentre os que cobrem maiores extensões podemos destacar as praias arenosas, os mangues e marismas. Nesses locais de transição entre o ambiente terrestre e o marinho e/ou estuarino, os organismos requerem grande plasticidade fisiológica devido principalmente aos parâmetros físico-químicos altamente variáveis (WILLIAMS & WILLIAMS 1998; BEREZINA 2003; SILBERBUSH *et al.* 2005; CHAINHO *et al.* 2006).

Mangues e marismas são ambientes conhecidos pela sua alta produtividade, pois a água que permeia esses ambientes carreia matéria orgânica vegetal para dentro do estuário/oceano, sustentando a trama trófica a partir do fitoplâncton (BOESCH & TURNER 1984; HUISKES 1988; COSTA 1998). Tais ambientes também funcionam como tanques armazenadores de carbono, conhecido como Ecossistemas de Carbono Azul (*Blue Carbon Ecosystems*; COPERTINO 2011), o que torna a fragmentação e o soterramento dessas áreas – ocasionados por diversos impactos antrópicos como a expansão das cidades e a agropecuária – uma problemática em termos de aquecimento global, efeito estufa e perda de diversidade (BERCHEZ *et al.* 2008; TCHAKONTÉ *et al.* 2015; YADAMSUREN *et al.* 2015). É evidente a predominância de ambientes de marismas em muitos países, principalmente em regiões estuarinas (COSTA *et al.* 2001); no entanto, há certa disjunção latitudinal, com mangues

frequentemente dominando em regiões equatoriais, enquanto marismas tendem a prevalecer em médias e altas latitudes (SILLIMAN 2014).

A fauna e flora dos ambientes de marisma são estruturadas principalmente por dois fatores: oscilação de maré e salinidade. As múltiplas combinações da variação desses fatores, juntamente à estratificação ocasionada pela distância ao nível do corpo d'água marinho ou estuarino, faz surgir um característico mosaico entre as espécies vegetais, denominadas marismas altas e marismas baixas. Marismas altas são caracterizadas pela presença de vegetação arbustiva e pela baixa frequência em que essas estão sujeitas ao alagamento. Contrário à isso estão as marismas baixas, dominadas por poucas espécies (ex.: Spartina spp.) e frequentemente alagadas (COSTA & MARANGONI 2010). No entanto, a oscilação de maré diária pode variar geograficamente: por exemplo, nas marismas da costa oeste dos EUA a oscilação é de 200-300 centímetros (PENNINGS & BERTNESS 2001), enquanto as marismas da costa do Golfo do México e do estuário da Lagoa dos Patos (STOUT 1984; COSTA 1998) experienciam variações de 20-40 cm. Nessas regiões com baixa oscilação mareal, a salinidade é o principal parâmetro abiótico que influencia a estruturação das marismas.

Em termos da fauna que ocupa as marismas, observamos uma mistura entre os grupos de origem marinha (bivalves, crustáceos, poliquetas) e de origem terrestre (aves, mamíferos, insetos). A natureza intermareal desse ecossistema controla a distribuição desses animais, mas diferindo em termos de fatores desses dois grupos: por exemplo, níveis baixos de alagamento favorecem os

animais terrestres, enquanto os níveis altos permitem a ocupação dos aquáticos nesse ambiente (PENNINGS & BERTNESS 2001)

A composição da fauna habitante das marismas pode ser influenciada pela sua posição no corpo estuarino e este pode ser dividido em três regiões dependendo de sua proximidade com o mar. O alto estuário recebe maior influência de águas dulcícolas, enquanto o baixo tem livre comunicação com o mar adjacente. Já o estuário médio apresenta mistura constante de águas doces e salgadas com rápidas variações de salinidade (THE OPEN UNIVERSITY 1999).

O complexo estuarino da Lagoa dos Patos (ELP), ao sul do Brasil, possui vastas áreas de marismas dominadas por poucas espécies de plantas: *Spartina alterniflora* e *Spartina densiflora* nos estratos inferiores e *Scirpus maritimus, Scirpus olneyi* e vegetação arbustiva (incluindo *Myrsine parvifolia*) nos superiores (COSTA *et al.* 2003). Algumas regiões recebem destaque, como as marismas da Ilha da Torotama, que são o limite superior do estuário (alto estuário). Esta ilha possui cerca de 40 km de largura e é coberta quase exclusivamente por marismas. Em uma posição mais central do estuário (médio estuário) está a Ilha da Pólvora, cujas marismas apresentam uma nítida estratificação entre as marismas alta e baixa. O limite das marismas mais próximo à desembocadura do estuário está localizada na Vila da Barra (no baixo estuário). A baixa oscilação de maré, junto à grande variação sazonal da precipitação (COSTA 1998), caracterizam essas marismas como irregularmente alagadas com condições hipersalinas durante o verão e oligo-mesohalinas no inverno (D'INCAO 1992).

As espécies vegetais de Spartina são dominantes dos estratos inferiores em marismas, e sustentam uma diversidade de organismos associados a suas raízes (CURADO et al. 2014) propiciada pela matéria orgânica vegetal abundante que favorece suspensívoros, decompositores e comedores de depósito (bivalves, crustáceos, poliquetas e insetos). PENNINGS & BERTNESS (2001) estimam que cerca de 10% do carbono fixado nesses ambientes fluem por estes organismos. Já nos estratos mais altos, há prevalência de arbustos e plantas arbóreas de pequeno porte. Os padrões ecológicos dos grupos de invertebrados em marismas já são bem conhecidos, exceto para insetos. É comum que Insecta seja inadequadamente agrupado com baixo refinamento taxonômico ou até como um único grande grupo (p.ex.: COSTA et al. 2001; MACKENZIE et al. 2015), o que dificulta o entendimento da sua ecologia uma vez que estes animais apresentam grande diversidade específica e ecológica. Para solucionar tal problema uma boa identificação morfológica aliada a ferramentas de identificação moleculares tornam-se cada vez mais necessárias (PILIPENKO et al. 2012). O método DNA barcoding, que envolve a análise de um fragmento padronizado do gene mitocondrial Citocromo Oxidase I (COI) é atualmente um dos métodos mais utilizados para a identificação de espécies animais, incluindo insetos (HEBERT et al. 2004).

Insecta é o grupo de animais mais diverso e abundante em marismas (CHENG 1976). Eles são, junto às aves, os únicos componentes desse ambiente capazes de contribuir diretamente no fluxo energético através da teia trófica, transferindo-a do ambiente aquático para o terrestre, uma vez que em muitas espécies a larva é aquática e o adulto é alado-terrestre (KELTZ, 1979). As ordens

Diptera, Coleoptera e Hymenoptera são as que mais se destacam em termos de diversidade e importância ecológica. As formigas e besouros possuem um importante papel ecológico na aeração das camadas superficiais do solo (PÉTILLON *et al.* 2008; DUMMEL *et al.* 2011; BOLICO *et al.* 2012), mas são dípteros (moscas e mosquitos) os insetos dominantes (KUBÁTOVÁ-HIRSŎVÁ 2005; MACKENZIE 2005; ROCHLIN *et al.* 2011). Dípteros são conhecidos principalmente por serem vetores de doenças, no entanto os hábitos alimentares desse grupo abrangem desde as larvas como decompositoras ou comedoras de depósito até os adultos fitófagos, succívoros, necrófagos ou polinizadores (GULLAN & CRANSTON 2007).

A influência dos parâmetros ambientais na flutuação da abundância de outros grupos de invertebrados é amplamente conhecida em marismas (NOLTE *et al.* 2013; MITWALLY & FLEEGER 2015), no entanto para insetos este conhecimento é escasso. Sabe-se que a abundância e riqueza dos insetos está diretamente relacionada com a sazonalidade, que é mais acentuada em zonas temperadas onde as estações são bem definidas (WOLDA 1988; SILVA *et al.* 2011; KISHIMOTO-YAMADA & ITIOKA 2015). Isso ocorre porque sua abundância está diretamente ligada à temperatura, umidade e precipitação (GONÇALVES-ALVIM & FERNANDES 2001; OLIVEIRA *et al.* 2006; CARRASCO *et al.* 2014). Os diferentes estágios larvais e de pupa tem sua distribuição espacial regida principalmente pela a matéria orgânica e o tamanho de grão sedimentar no ambiente terrestre (PETERSEN *et al.* 2013). A intensidade do vento também é uma importante variável, pois pode exercer o carreamento dos alados para outras áreas ou forçar o pouso, principalmente

para espécies de grande porte e envergadura de asa, como os Tipuloidea (3-36 mm; GELHAUS 2009).

Tipuloidea é a maior superfamília dentre os mosquitos com 15.437 espécies atualmente descritas (OOSTERBROEK 2016), e estão geralmente associados a ecossistemas úmidos como margens de corpos d'água. As suas larvas aquáticas ou semi-aquáticas ocupam uma larga variedade de habitats. GELHAUS (2009) lista alguns gêneros e respectivos habitats:

- ✓ Dicranomyia: zonas intermareais ou água salobra;
- ✓ Atarba, Teucholabis, Lipsotrhrix: madeira em decomposição;
- ✓ Molophilus: terra ou lodo rico em matéria orgânica;
- Toxorhina, Rhipidia: folhas e caules em vários estágios de putrefação;
- ✓ Geranomyia: plantas terrestres;
- ✓ Nephrotoma, Tipula: solos ricos em húmus e pastagens.

A pupa dos tipulídeos é comumente encontrada no mesmo micro-habitat larval, e os adultos podem ser encontrados em repouso na vegetação adjacente ao corpo d'água. Esse conjunto de características fazem das marismas um ecossistema berçário para essa família. A fase adulta é relativamente curta comparado à larva, e muitas espécies não se alimentam nesta fase, usando-a apenas para dispersão, voo nupcial e oviposição. Esse curto período permite melhor avaliar a resposta dos tipulídeos a fatores abióticos e entender suas preferências de voo e reprodução. Além disso, a maioria das espécies de tipulídeos adultos possuem picos de abundância no verão e na primavera, períodos mais quentes e úmidos (FREEMAN 1968; FREEMAN & ADAMS 1972; PRITCHARD 1983). No entanto, outras se estabelecem durante o inverno, podendo inclusive serem capazes de tolerar o congelamento (VANIN & MASUTTI 2008; TASCHEREAU *et al.* 2009; HÅGVAR *et al.* 2010).

O entendimento da distribuição e diversidade de Tipuloidea para as diferentes zonações das marismas e do gradiente estuarino, assim como os principais fatores abióticos que regem a flutuação de sua abundância, são importantes para a compreensão do papel biológico e ecológico exercido pelo grupo nesse ambiente. Dessa forma, o presente estudo caracterizou a assembleia de tipulídeos frente a parâmetros espaciais, temporais e abióticos.

OBJETIVO GERAL

Utilizar ferramentas moleculares para identificação e filogenia das espécies de Tipuloidea, além de avaliar a distribuição espacial e temporal das mesmas e suas respostas a parâmetros ambientais em marismas do estuário da Lagoa dos Patos.

OBJETIVOS ESPECÍFICOS

- Identificar as espécies de Tipuloidea através de características morfológicas e ferramentas moleculares (DNA *barcoding*), resultando em um *checklist*,
- Desenvolver uma chave de identificação para os gêneros de Tipuloidea que ocorrem nas marismas do ELP;
- Desenvolver uma árvore filogenética entre as espécies de Tipuloidea através de amplificação e comparação da região Citocromo Oxidase I do DNA;

- Avaliar a variação espacial e temporal na composição, abundância, riqueza e diversidade dos adultos de Tipuloidea entre diferentes pontos amostrais, estratos de marisma, e estações do ano;
- Verificar as flutuações sazonais de adultos e estabelecer suas correlações com variáveis ambientais.

MATERIAL E MÉTODOS

Área de estudo

O estudo foi realizado em três marismas adjacentes ao complexo estuarino da Lagoa dos Patos (Fig. 1). No sentido lagoa-oceano o primeiro ponto amostral é na Ilha da Torotama (31° 55' 9.649" S; 52° 8' 25.300" O), o segundo mais centralizado ao estuário na Ilha da Pólvora (32° 1' 19.538" S; 52° 6' 17.971" O), e o terceiro na Vila da Barra, no baixo estuário (32° 9' 13.028" S; 52° 6' 21.629" O).



Figura 1. Área de estudo com os 3 pontos amostrais nas marismas ao longo do Estuário da Lagoa dos Patos.

Nos três pontos amostrais o estrato de marisma alta é coberta por diversos tipos vegetais incluindo arbustos e árvores de diversas espécies, mas com maior presença de *Myrsine parvifolia* (Fig. 2). Nesse ambiente há presença de aves pequenas, uma menor influência de vento resultante da presença de vegetação arbustiva, serapilheira, e matéria orgânica em decomposição. Já no estrato de marisma baixa, há uma maior constância de ventos, por vezes com menor intensidade e em outras com fortes rajadas mesmo em períodos quentes como o verão. Nesse estrato há presença exclusiva de *Spartina alterniflora* e *S. densiflora*, as quais são plantas herbáceas halófitas que caracterizam esses ambientes.

Amostragem

Para a coleta dos Tipuloidea foram confeccionadas seis armadilhas tipo Malaise de 1,50 m de altura por 1,60 m de comprimento (Fig. 2). Foram instaladas duas armadilhas por ponto amostral (uma por estrato de marisma). Esse método de captura funciona por interceptação de voo e é ideal para estudos em comunidade e assembleias de insetos alados uma vez que há baixa seletividade na captura desses animais, gerando maior confiabilidade na captura majoritária dos representantes do ambiente estudado (CONTRERAS-RAMOS & GELHAUS 2002). As armadilhas permaneceram em campo durante 12 meses (de agosto/2015 a julho/2016) e o copo coletor contendo álcool 70% foi trocado a cada 15 dias. Algumas amostras foram perdidas devido a ventos e tempestades, levando a um total de 107 amostras (38 Ilha da Torotama, 38 Ilha da Pólvora, 31 Vila da Barra). Os insetos coletados foram levados ao Laboratório de Crustáceos Decápodes para triagem e identificação com auxílio de lupa e microscópio estereoscópico, e posteriormente armazenados e etiquetados em micro-tubos. A amostragem foi realizada sob licença SISBIO número 50253-1.



Figura 2. Armadilha Malaise instalada no estrato de marisma alta, próxima a arbustos de *Myrsine parvifolia* (A); Detalhe no copo coletor (B).

Identificação morfológica

A identificação morfológica genérica foi realizada segundo ALEXANDER & BYERS (1981) e GELHAUS (2009) e específica por ALEXANDER (1912a, 1912b, 1913a, 1913b, 1927, 1935, 1937a, 1937b, 1938, 1945 e 1969) e ANDREW (2000). Os insetos identificados foram depositados junto à coleção da Fundação Zoobotânica do Rio Grande do Sul – FZB/RS.

Identificação molecular e filogenia

O DNA genômico de três exemplares de cada espécie foi extraído através do método fenol/clorofórmio ou salino adaptado de SAMBROOK *et al.* (1989) e ALJANABI & MARTINEZ (1997), com o auxílio de maceração inicial com nitrogênio líquido para quebra de quitina dos exemplares. O DNA foi avaliado qualitativamente através eletroforese em gel de agarose 1% e quantificado através do espectrofotômetro BIODROP.

Reações em cadeia da polimerase (PCR) foram realizadas em um termociclador para amplificação do gene Citocromo Oxidase I (COI), utilizando os iniciadores universais LCO1490: 5'-GGGTCAACAAATCATAAAGATATTGG-3' e HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (FOLMER *et. al.* 1994) e com concentrações de reagentes adaptadas de PILIPENKO *et al.* (2012): 4.0 ng de DNA; 2.5 U Taq DNA polimerase (Ludwig Biotec); 0.4 Mm Dntp mix; 3 Mm MgCl2; e 10% de buffer, em um volume total de 25 µl. As condições de reação foram: 1 min à 94°C, 35 ciclos de 1 min à 94°C, 1 min à 51°C e 2 min a 72°C, seguido por um passo de extensão final de 5 min à 72°C e 4°C até a retirada do material do equipamento.

O material amplificado foi purificado através da precipitação com Polietileno Glicol (PEG) 8000 15% (HARTLEY & BOWEN 1996), e ressuspenso em tampão TE. Após verificação em gel de agarose, os produtos purificados foram sequenciados na empresa Macrogen (http://dna.macrogen.com/eng/) e ACTGene. As sequências resultantes foram alinhadas e editadas no programa BioEdit 7.0, e os haplótipos obtidos foram comparados com sequências disponíveis em bancos dados públicos (GenBank e BOLD) para corroborar as

identificações morfológicas. As espécies foram consideradas identificadas apenas quando houve 97% de correspondência com as espécies já descritas. Para haplótipos não descritos ou divergentes dos disponíveis nos bancos de dados, foram construídas árvores para avaliar as relações filogenéticas da assembleia. As árvores foram geradas através do método de máxima verossimilhança e a confiabilidade das filogenias geradas foram avaliadas através de testes *bootstrap* (10000 repetições), implementadas no programa R e utilizando os pacotes "ape" e "phytools".

Dados abióticos

Os parâmetros temperatura do ar instantânea (°C), umidade relativa do ar instantânea (%), velocidade do vento (m/s) e precipitação (mm/h) foram obtidos pelo Instituto Nacional de Meteorologia (INMET, 2016). Estes dados são medidos de hora em hora na estação meteorológica automática Rio Grande-A802 (localizada na vila da Barra; latitude: 32° 8' 54.438" S e longitude: 52° 6' 12.452" O), planilhados e disponibilizados através da plataforma online. Os dados de salinidade foram cedidos pelos pesquisadores responsáveis pelo projeto "Monitoramento das espécies invasoras no estuário da Lagoa dos Patos". (Sigproj: 2112511.983.137230.290720015) do Laboratório de Crustáceos Decápodes – FURG, os quais coletavam alíquotas de água diariamente em pontos próximos aos das armadilhas Malaise.

Análises estatísticas

I) Eficiência de coleta

Para avaliação da eficiência de coleta das espécies de Tipuloidea, foi elaborada uma curva de acumulação de espécies usando combinações randômicas entre as unidades amostrais para os três pontos amostrados. Médias e erro padrão foram calculadas usando o método Mao-Tau (COLWELL *et al.* 2004). Adicionalmente, foi estimada a riqueza de espécies para cada ponto amostral e para o conjunto dos pontos coletados usando o método CHAO e Jackknife de primeira ordem, estimadores ideais para dados de abundância (GOTELLI & COLWELL 2011).

II) Frequência de ocorrência

As espécies foram classificadas de acordo com sua frequência de ocorrência segundo DAJOZ (1974) como constantes (>50% das coletas), acessórias (entre 25 e 50%), acidentais (<25%) e ausentes.

III) Composição, riqueza e estrutura da assembleia

Para melhor compreender a estruturação da assembleia de tipulídeos em termos de Riqueza e Equabilidade nas diferentes estações do ano, foi desenvolvido um Diagrama de Whitakker (MELO 2008), onde no eixo do Y está notada a abundância relativa enquanto no eixo do X estão notadas todas as espécies em ordem decrescente de abundância. O cálculo da abundância relativa é mostrado abaixo:

$$Ar = \frac{Ai * 100}{N}$$

Onde:

Ar = Abundância relativa

Ai = Abundância absoluta

N = total de indivíduos capturados

Uma análise de coordenadas principais (PCoA) foi gerada baseada em uma matriz de dissimilaridade de Bray-Curtis (ideal para dados de contagem) utilizando os pacotes "vegan" e "ape" implementados no software R. Para testar as diferenças espaciais e temporais da assembleia de tipulídeos ainda foi realizada uma Análise de Variância Permutacional Multivariada (PERMANOVA) utilizando os dados de abundância. O modelo utilizado compreendeu os fatores pontos (fixo – 3 níveis), estratos de marisma (fixo – 2 níveis) e estações do ano (fixo – 4 níveis). A PERMANOVA possui a mesma robustez que a ANOVA, mas contrário à essa última, não requer os pressupostos normalidade. Apesar de não apresentar um pressuposto de homogeneidade de variâncias, a análise pode ser sensível a ampla dispersão entre os grupos (ANDERSON 2001; ANDERSON 2005). A análise foi baseada em uma matriz de dissimilaridade de Bray-Curtis onde os dados foram permutados 9999 vezes, e foi implementada no programa R, com a função *adonis()* do pacote "vegan".

IV) Influência abiótica

Para avaliar quais parâmetros abióticos (temperatura, umidade do ar, velocidade do vento, precipitação e salinidade) tiveram correlação com a abundância das diferentes espécies, foi elaborada uma Análise de Correspondência Canônica (CCA) seguida de ANOVAs para averiguar a confiabilidade da análise utilizando as funções *cca()* e *anova.cca()*. Nessa análise foram excluídas as espécies raras (menos de 10 indivíduos). Para todas as análises foi usado um nível de significância de 5% (p-valor < 0.05).

SÍNTESE DOS RESULTADOS

CAPÍTULO I: *Checklist*, chave de identificação e relações filogenéticas baseadas em mtDNA (COI) das espécies de tipulídeos (Diptera: Tipuloidea) de marismas sul Neotropicais

Foram capturados ao todo 5.248 espécimes de Tipuloidea distribuídos em 16 espécies. Das quatro famílias de Tipuloidea (Cylindrotomidae, Limoniidae, Pediciidae and Tipulidae *stricto sensu*) apenas as duas maiores – Limoniidae e Tipulidae – ocorreram nas marismas. Além disso, cinco morfotipos (todos pertencentes à Limoniidae) dos gêneros *Geranomyia* Haliday 1833, *Ormosia, Molophilus* Curtis 1833 e outro sem identificação em nível de gênero, e um complexo de espécies de *Dicranomyia*, também foram observados. O gênero *Ormosia* teve seu primeiro registro para a região Neotropical e a distribuição das espécies *Polymera inornata* e *Symplecta cana* foi ampliada para todo continente

Americano. Uma chave de identificação ilustrada para guinze gêneros que ocorrem nas marismas do Estuário da Lagoa dos Patos foi elaborada. As relações filogenéticas corroboraram 'Limoniidae' como um agrupamento artificial, enquanto Tipulidae stricto sensu manteve-se como uma família monofilética. O agrupamento das espécies sob nível das subfamílias Chioneinae, Limoniinae e Limnophilinae para 'Limoniidae' e da subfamília Tipulinae para Tipulidae stricto sensu mostra-se como a melhor maneira de classificação dentro de Tipuloidea. Apesar de Limoniinae em geral ser monofilético, as espécies dos gêneros Dicranomyia e Geranomyia não demostraram essa premissa. Chioneinae, assim como apoia a literatura, mostrou-se um agrupamento artificial. Nossos resultados, assim como outros estudos, apontaram Tipulinae como um grupo monofilético. A listagem das espécies juntamente com a discussão da ampliação de suas distribuições e relações filogenéticas estão no artigo "Checklist, identification key and phylogenetic relationship based on mtDNA (COI) of craneflies species (Diptera: Tipuloidea) from southern neotropical salt marshes", submetido à revista *Neotropical Entomology* – Qualis B2 (CAPES – Biodiversidade). Este artigo pode ser encontrado no Apêndice I da dissertação.

CAPÍTULO II: Estruturação espaço-temporal e influência de parâmetros abióticos na assembleia de Tipuloidea (Insecta: Diptera) em marismas Neotropicais

Ao longo de um ano de amostragem (agosto/2015 a julho/2016) foram coletados 5.248 indivíduos de tipulídeos. Além das 16 espécies identificadas a nível específico da seção anterior, outras 15 morfoespécies foram inclusas. A abundância, abundância relativa e frequência de ocorrência das espécies foram avaliadas para os três pontos amostrais. A curva de acúmulo de espécies revelou maior riqueza no estuário superior seguido do estuário inferior e médio. O método e período de amostragem foram considerados adeguados considerando o intervalo de confiança expresso pelos estimadores de riqueza. Seis e quatro espécies foram consideradas constantes no estuário superior e inferior, respectivamente. Uma lacuna no médio estuário foi observada, com apenas uma espécie Symplecta pilipes. Observou-se ainda um gradiente das espécies acessórias no sentido lagoa-oceano: sete, quatro e dois. Três espécies foram exclusivas, uma do alto estuário e duas do baixo. A PERMANOVA detectou diferença na abundância entre os pontos amostrais, estações do ano e estratos de marisma. A diferença temporal entre as estações do ano ainda foi bem representado pela PCoA, separando claramente inverno e verão. Outono e primavera tiveram certo grau de similaridade entre si e com as outras estações. A abundância na marisma alta foi maior que na baixa. Por último, a CCA detectou a presença de três grupos dentro da assembleia de tipulídeos, com dois deles esperados e corroborados pela literatura e o terceiro revelando S. pilipes como

uma espécie antagonista de clima mais frio. As análises gráficas e discussão desses resultados estão aprofundadas no segundo manuscrito da dissertação, correspondente ao Apêndice II. Este artigo tem título de "Spatial-temporal structuring of Tipuloidea (Insecta: Diptera) assemblages at Neotropical salt marshes, and influence of abiotic parameters", e será submetido à revista *Journal of Animal Ecology* – Qualis A1 (CAPES – Biodiversidade).

CONCLUSÕES

- Foram identificadas 31 tipulídeos entre espécies e morfoespécies, pertencentes à Limoniidae e Tipulidae *stricto sensu*. Um complexo morfológico de espécies de *Dicranomyia* e uma possível nova espécie de Tipuloidea foram registrados nesse estudo.

 Uma chave de identificação para os quinze gêneros de Tipuloidea foi desenvolvida, e poderá ser utilizada em outros estudos acerca desse grupo de mosquitos em marismas.

 A identificação molecular não foi possível através dos bancos de dados online devido a dois motivos: 1) baixa similaridade entre as sequências disponibilizadas no sistema e as geradas por esse estudo; e 2) ausência de maior refinamento taxonômico quando havia alto grau de similaridade;

 A árvore filogenética corroborou Tipulidae strictu senso como um grupo monofilético. Apesar de Limoniinae em geral ser monofilético, as espécies dos gêneros Dicranomyia e Geranomyia não demostraram essa premissa.
 Chioneinae, assim como apoia a literatura, é um agrupamento artificial;

 A amostragem de Tipuloidea no Estuário da Lagoa dos Patos foi satisfatório, estando o número de espécies observadas dentro dos limites dados pelos estimadores de riqueza;

 A lacuna de espécies constantes na Ilha da Pólvora (médio estuário) pode ser atribuído à maior variação da salinidade nesse ponto, ocasionando estresse e impedindo a alocação das espécies de tipulídeos;

 Tipulídeos tiveram maior abundância nos estratos de marisma alta devido à presença de vegetações arbustivas, que possibilitam aos insetos pousarem, copularem, e se alimentarem, além de fornecer sombreamento;

- Em escala temporal houve uma presença alternada entre as espécies de tipulídeos: durante o inverno houve 80% dominância de *Symplecta pilipes*, enquanto no verão foi observado maior equabilidade variando de 15 a 20% entre outras espécies como *Dicranomyia* complex, *Geranomyia recisa*, *Rhipidia domestica amazonensis* e *Symplecta cana*;

 As possíveis consequências ecológicas dessa alternância incluem a troca constante das espécies que compõem a guilda detritívora e/ou succívora exercida pelos tipulídeos, afetando os níveis tróficos superiores, como aves e peixes insetívoros que habitam marismas;

 Os dados de ocorrência, distribuição e flutuação da abundância de tipulídeos permite melhor compreender seu papel trófico e ecológico em marismas.
 Sugere-se que Tipuloidea, juntamente com os demais grupos de insetos, são parte importante da fauna integrante desse ecossistema.

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crane flies (Diptera: Tipuloidea) indicate impact from grazing by livestock in the Hövsgöl region of Mongolia. *Journal of Insect Conservation*, 19: 465– 477. **APÊNDICE I**

CHECKLIST, IDENTIFICATION KEY AND PHYLOGENETIC RELATIONSHIP BASED ON mtDNA (COI) OF CRANEFLIES SPECIES (DIPTERA: TIPULOIDEA) FROM SOUTHERN NEOTROPICAL SALT MARSHES

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ABSTRACT

Craneflies (Tipuloidea) are the most diverse group within order Diptera. Considering the scarcity of information on biology and ecology of this group at Neotropics, and the sparse literature available for species identification, we developed a checklist using morphology and molecular methods, and a generic identification key for craneflies sampled at South Brazilian salt marshes. Two malaise traps in each one of three points were installed along Patos Lagoon Estuary, Rio Grande do Sul state, from August 2015 to July 2016. Collector cups were changed every fifteen days, and collected flies were separated, identified and deposited at the Rio Grande do Sul Zoobotanic Foundation (Fundação Zoobotânica do Rio Grande do Sul - FZB/RS). A total of 16 species and three morphotypes belonging to Limoniidae and Tipulidae stricto sensu were identified. Distribution ranges of Symplecta cana (Walker, 1848) and two Ormosia Rondani, 1856 species were expanded. Additionally, the *Dicranomyia* Stephens, 1829 species complex was recorded. An identification key with thirteen genus was elaborated. The phylogenetic relations between subfamilies confirm that Tipulidae stricto sensu is a monophyletic group, while Limoniidae is an artificial grouping. This is one of the first studies to report the occurrence and distribution of crane flies at the Neotropical region, and will serve as a basis for future research on Tipuloidea.

KEY WORDS: Coastal insects; coastal ecosystem; tipuloids; distribution range; identification key

INTRODUCTION

The Tipuloidea superfamily is considered the richest within order Diptera. It is subdivided into Cylindrotomidae, Limoniidae, Pediciidae and Tipulidae *stricto sensu*. Approximately 3.500 cranefly species are recognized throughout the Neotropical region, of which around 99.6% are included in families Limoniidae and Tipulidae (De Jong et al., 2008, Oosterbroek, 2016). Craneflies are non-hematophagous flies that are extremely important in the trophic webs they participate in, presenting a detritivorous diet during their larval phase and adopting a succivorous and/or pollinizing phytophagous diet as adults. This group is widely distributed over almost all ecosystems, and includes species that occupy wetlands such as salt marshes (Rogers, 1932).

Salt marshes are typical coastal environments of mid- and high-latitude areas, including the southern Neotropical region. They are characterized as transition areas between estuaries and land, display few but dominant plant species, and require fauna and flora that tolerate fast variations in salinity (Costa and Marangoni, 2010). The salt marshes of the Patos Lagoon Estuary are one of the largest in Brazil, covering over 70 km² (Costa et al., 1997). Mean temperature at the area is 22.7 °C, with mean precipitation of 104 mm³/month and wind velocity of 3.03 m/s (INMET, 2016). Estuarine flow and precipitation levels tend to increase salinities at these salt marshes during the summer, and decrease in the winter (D'Incao et al., 1992). The margins of this estuarine environment are dominated by halophyte plant species such as *Spartina alterniflora* and *S. densiflora*, as well as *Myrsine parvifolia* shrubs (Costa et al., 1997).

Although several Tipuloidea have been described by C. P. Alexander, few studies have reported and updated the occurrence of cranefly species across the Neotropical region (see Ribeiro et al., 2007; Ribeiro and Santos, 2016), with most records of this group having been reported for the Holoarctic region (Ebejer, 2015; Podenas et al., 2015; Starý and Čelechovský, 2015). Additionally, researches at salt marshes are commonly focused on species such as fish, polychaetes and crustaceans, but little to no studies on the identification of insect groups are conducted at these environments (e.g. Contente et al., 2010; Campos et al., 2015; MacKenzie et al., 2015). It is necessary to identify and report the insect species that inhabit these environments, preferably with high taxonomic refinement, in order to support future studies on classification, biogeography and ecology of these groups. The large corporal mass of craneflies, along with its occupation of transitional ecosystems (e.g. salt marshes), makes these insects an important trophic link between aquatic and terrestrial environments. In this manner, this study aimed to identify (through morphological and molecular methods) and list the Tipuloidea species that occur at salt marshes of the southern Neotropical Region, and develop an identification key of the group.

METHODS

Study area and sampling. Three salt marsh areas of the Patos Lagoon Estuary were selected: high estuary (31°53'33"S; 52°14'33"W), middle estuary (32°02'01''S; 52°10'45"W), and low estuary (32°10'65"S; 52°08'52"W) (Fig. 1). Two Malaise traps with ethanol-filled collection cups were installed at each sampling point, close to the dominant vegetation, in a total of six traps. These traps function by intercepting flight, and are known for their lack of selectivity in capturing winged insets, including craneflies (Contreras-Ramos and Gelhaus, 2002). Sampling was conducted over twelve months,

from August 2015 to July 2016. Collection cups were changed every fifteen days, and collected insects were taken for identification and preservation in 70% ethanol. Specimens were deposited at the Rio Grande do Sul Zoobotanic Foundation (*Fundação Zoobotânica do Rio Grande do Sul -* FZB/RS). Sampling was conducted under SISBIO license number 50253-1.



Figure 1. Sampling areas at salt marshes of the Patos Lagoon Estuary, in South Brazil.

Morphological identification. Genus identification was based on Alexander and Byers (1981) and Gelhaus (2009), while species identification was based on Alexander (1912a, 1912b, 1913a, 1913b, 1927, 1935, 1937a, 1937b, 1938, 1945 and 1969), Andrew (2000), and Starý and Brodo (2009).

Species distribution. Description of species distributions was based on the *Catalogue of the craneflies of the World* (Oosterbroek, 2017). However, we highlight that this

catalogue does not differentiate between different regions within Brazil, which is important for such a large country with pronounced regional variations.

DNA extraction, amplification and sequencing. Genomic DNA of three specimens of each species was extracted through phenol:chlorophorm or salt extraction protocols adapted from Sambrook et al. (1989) and Aljanabi and Martinez (1997), with initial cryogenic grinding with liquid nitrogen for breakdown of chitin DNA quantity and quality was assessed through electrophoresis with 1% agarose gel and a BIODROP spectrophotometer. Polymerase Chain Reactions (PCR) were conducted in a Veriti thermocycler to amplify approximately 690 bp of the Cytochrome Oxidase I (COI) gene of mitochondrial DNA. using universal primers LCO1490: 5'-GGGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et. al. 1994), using reaction conditions adapted from Pilipenko et al. (2012): 4.0 ng DNA; 2.5 U Taq DNA polymerase (Ludwig Biotec); 0.4 Mm dNTP mix; 3 Mm MgCl2; and 10% buffer, in a final volume of 25 µl. Cycling conditions were: 1 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 51 °C and 2 min at 72 °C; followed by a final extension of 5 min at 72°C. Amplified products were purified with Polyethylene Glycol (PEG) 8000 15% (Hartley and Bowen, 1996) and ressupended in TE buffer. Purified products were sequenced in both directions at Macrogen (http://dna.macrogen.com/eng/).

Molecular identification. Sequences were visually checked for errors, edited and aligned using BioEdit 7.2.5, and compared to public online databases (Genbank and BOLD) for identification. Since most of the detected haplotypes were missing or divergent from the ones in the databases, we constructed a tree to evaluate the phylogenetic relations of the assemblage. The tree was generated through the Maximum Likelihood method, and its

reliability was evaluated through a bootstrap test (10000 repetitions), using the "ape" and "phytools" packages in R software.

RESULTS

A total of 5,248 specimens distributed over 16 craneflies species were captured at the salt marshes of Patos Lagoon Estuary. Three belong to family Tipulidae and the other 13 to Limoniidae. No Cylindrotomidae or Pediciidae specimens were observed. Furthermore, five Limoniidae morphotypes of genera *Geranomyia* Haliday 1833, *Ormosia*, *Molophilus* Curtis 1833, and one without genus identification, as well as a *Dicranomyia* species complex, were also observed. *Symplecta pilipes pilipes* (Fabricius, 1787) and *Rhipidia domestica amazonensis* Osten Sacken, 1860 represented 68% of all sampled craneflies. All species of the checklist are listed in Table 1, and the generic identification key is presented below.

Tipuloidea genus identification key, modified from Gelhaus 2009

1 - Well developed rostrum, longer than the remainder of the head, usually with anteriorly
projecting and sharply pointed nasusTipulidae 2
1' - Short rostrum and nasus absent, but occasionally lengthened as in Geranomyia and
ToxorhinaLimoniidae 4
2 – Branched antennae. Flagellomeres 2-7 each with branchesOzodicera
2' – Antennae without branched flagellomeres
3 – Vein Sc ending nearly opposite of origin of Rs; Rs short, oblique; cell dm with almost
equilateral veins; bm-cu present

3' - Vein Sc longer, ending after the origin of Rs; a single side of dm cell shorter,
sometimes conspicuous; bm - cu absentZelandotipula
4 – Head with long and slender rostrum and mouthparts, greatly exceeding length of head,
usually half of body length or more
4' - Head with non-elongated rostrum and mouthparts; maximum length as long as
head
5 – Fused mouthparts <i>Toxorhina</i>
5' – Loose mouthpartsGeranomyia
6 – Rostrum as long as the remainder of head <i>Teucholabis</i>
6' – Rostrum conspicuous or absent7
7 – Antennae of males as long as or larger than the entire bodyPolymera
7' – Antennae of males and females shorter than the entire body
$8 - R_2$ absent
$8' - R_2$ present
9 – Cell dm openedGonomyia (Neolipophleps)
9' – Cell dm closed; R ₄₊₅ very close to M ₁₊₂ Gonomyia (Paralipophleps)
10 – Cell dm opened
10' – Cell dm closed
$11 - R_4$ and R_5 beginning after bt CuA ₁ <i>Molophilus</i>
$11' - R_4$ and R_5 beginning nearly opposite the origin of bt CuA ₁ Ormosia

12 – Antennae with 12 flagellomeres	13
12' – Antennae with 13-14 flagellomeres	14
13 – Flagellomeres of males and females unipectinate	or subpectinate
	Rhipidia
13' – Flagellomeres of males and females oval to elongated shape	Dicranomyia
14 – A ₂ with "S" shape at midlength; Supernumerary cross	wein in cell r ₃
Symp	electa (Symplecta)
14' – A ₂ straight up to wing marginSyr	nplecta (Trimica)

Specimens used in the molecular phylogeny are presented in Table 2. Apart from specimens presented in the checklist, we also included the two *Ormosia* morphotypes, the *Geranomyia* morphotype and the limoniid without genus identification (indicated as Unknown genus). Species *Toxorhina brasilienses*, *T. meridionales* and *Ozodicera cinereipennis* were not included due to the low number of sampled individuals.

Phylogenetic analysis showed that 'Limoniidae' cannot be considered as monophyletic group, since specimens from this family were grouped at two distinct sections of our tree. Subfamily Chioneinae had distinct groupings: one of (*Gonomyia condensa* + *Teucholabis* sp), one of *Geranomyia* (a Limoniinae) + *Gonomyia pleuralis*, and finally one composed of *Ormosia*, *Symplecta* and a species of unknown genus ((((*Ormosia* sp1 + *Ormosia* sp2) + *Symplecta cana*) + unknown species and genus) + *Symplecta pilipes*). *Polymera obscura* and *P. inornata*, the only representatives of Limnophilinae, grouped with the outgroup *Aedes*. *Zelandotipula ringens* and *Nephrotoma triobtusa* grouped with the

outgroup *Tipula paludosa*: ((*Tipula paludosa* + *Zelandotipula ringens*) + *Nephrotoma triobtusa*), all belonging to monophyletic Tipulidae stricto sensu. With the exception of *Geranomyia*, species of the Limoniinae subfamily ((*Dicranomyia miseranda* + *Rhipidia domestica amazonensis*) + *Dicranomyia* complex) grouped at the base of the tree close to the *Drosophila melanogaster* outgroup, indicating paraphily to the other species.



Figure 2. Neighbor-joining tree based on Cytochrome oxidase I sequences. Purple lines represent outgroups.

DISCUSSION

The richness of insects in salt marsh environments is commonly underestimated due to the general assumption that this environment is hostile for insects with aquatic/semiaquatic larval phases, especially due to high salinity levels. However, previous studies have shown that salinity does not influence the presence of Diptera species in these habitats (Williams and Williams, 1998; Giberson et al., 2001; MacKenzie, 2005; Silberbush et al., 2005; Boix et al., 2008), which is also evidenced by our results. The majority of species identified at the studied salt marshes belong to Limoniidae, corroborating De Jong et al. (2008), who reports this family as the richest of the Tipuloidea.

Some of the groups we identified are noteworthy: *Dicranomyia* is the largest Limoniidae genus, representing around 10% of Neotropical species. Due to the fact that its representatives display some morphological differences, it is common for these insects to be treated as a species complex (Nitta and O'Grady, 2008; Goodman and O'Grady, 2013; Salmela et al., 2014). Therefore, the species within *Dicranomyia* complex were all genetically similar, which suggests that their different morphologies, especially in terms of wing venation, are a morphological plasticity of the group. This is the first report of genus *Ormosia* for the Neotropical region, and consequently for Brazil. We expanded the *Polymera inornata* Alexander, 1913 and *Symplecta cana* range record, with the latter for the entire American continent. *Symplecta pilipes* is a cosmopolitan species, so its presence was expected, and combined with *Rhipidia domestica amazonensis* represented 68% of all sampled craneflies.

As suggested by several authors, Tipulidae *stricto sensu* was shown to be a monophyletic group (Ribeiro, 2008; Petersen et al., 2010). Our study corroborated the current classification of Limoniidae as an artificial grouping (Petersen et al., 2010), likely due to *Dicranomyia* and *Geranomyia* within Limoniinae, since they appear separately in our tree. Ribeiro (2008) considered Limnophilinae paraphyletic to Tipulinae, which was also observed here. The Chioneinae subfamily showed at least two origins in our tree; this result does not corroborate Petersen et al. (2010), who describe this group as strongly supported across both molecular and morphological analyses. Additional studies using additional molecular markers are necessary to clarify the phylogeny of this group.

The current literature on Tipuloidea at the Neotropical region is sparse and fragmented, and presented mostly by C. P. Alexander. The majority of listed species have also been recorded in Central America, confirming their wide latitudinal occurrence; others are more restricted and occur only in Southeast/South Brazil, possibly due to a strong association with the studied ecosystem. This is the first work to list the Tipuloidea species of Neotropical salt marshes in South Brazil, decreasing the large gap in information on craneflies, and aiding in future studies aimed towards exploring the life cycles and/or ecology of these insects.

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Table 1. List of species, references, and previous distribution records of craneflies found in salt marshes of the Patos Lagoon Estuary.

DEPOSIT			
NUMBER	TAXA	REFERENCES	PREVIOUS DISTRIBUTION *
	LIMONIIDAE		
69853	Dicranomyia complex Stephens1829	-	-
69856	Dicranomyia (Dicranomyia) miseranda (Alexander, 1945)	Alexander, 1945	Brazil (Southeastern region)
69842	Geranomyia recisa Alexander, 1927	Alexander, 1927	Mexico, El Salvador, Panama, Ecuador, Brazil (Southeastern region), Peru
69854	Gonomyia (Neolipophleps) condensa Alexander, 1938	Alexander, 1938	Brazil (South region)
69852	Gonomyia (Paralipophleps) pleuralis (Williston, 1896)	Alexander, 1912a	USA, Bermuda, Cuba, Haiti, Dominican Republic, Puerto Rico, Costa Rica (Isla de Coco), Dominica, Trinidad, Guyana, Peru, Bolivia, Brazil (Southeastern region), St. Vincent
69844	Polymera (Polymera) obscura Macquart, 1838	Alexander, 1913a	Mexico, Panama, Guyana, Peru, Bolivia, Brazil (North region), Argentina
69845	Polymera (Polymera) inornata Alexander, 1913a **	Alexander, 1913a	Guiana

69843	Rhipidia domestica amazonensis Osten Sacken, 1860	Alexander, 1912b	Brazil (North region)
69857	Symplecta (Symplecta) cana (Walker, 1848) **	Starý and Brodo, 2009	Canada, USA (Alaska, Yukon and NWT to Nfld, South throughout the USA), Mexico, Guatemala
69849	Symplecta (Trimica) pilipes pilipes (Fabricius, 1787)	Andrew, 2000	The only cosmopolitan cranefly, widespread in all biogeografical regions (Driauach and Belqat, 2016)
69848	Teucholabis (Teucholabis) fuscoapicalis Alexander, 1937	Alexander, 1937a	Brazil (South region)
69855	Toxorhina (Toxorhina) brasiliensis (Westwood, 1836)	Alexander, 1913b	Brazil (North and Southeastern region)
69846	Toxorhina (Toxorhina) meridionales Alexander, 1913	Alexander, 1913b	Brazil (North region)
	TIPULIDAE		
69847	Nephrotoma triobtusa Alexander, 1969	Alexander, 1969	Brazil (South region)
69850	Ozodicera (Ozodicera) cinereipennis Alexander, 1937b	Alexander, 1937b	Brazil (Southeastern region)
69851	Zelandotipula ringens (Alexander, 1935)	Alexander, 1935	Paraguay, Brazil (Southeastern region)

* As described in Oosterbroek (2017)

** First record in Brazil

1	APÊNDICE II
2	
3	SPATIO-TEMPORAL STRUCTURE AND INFLUENCE OF
4	ENVIRONMENTAL PARAMETERS ON THE TIPULOIDEA (INSECTA:
5	DIPTERA) ASSEMBLAGE OF NEOTROPICAL SALT MARSHES
6	
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19	
20	Abstract
21	1. Estuaries and salt marshes are important coastal ecosystems that present unique
22	characteristics in terms of nutrient cycling, salinity, habitats, flora and fauna.

Despite their ecological importance, knowledge on the occupation is scarce,
distribution and ecology of insects, including Tipuloidea, in these environments.

- 25 2. This study aimed to evaluate the composition, seasonality and effect of abiotic
 26 factors on the abundance, diversity and structure of a Tipuloidea assemblage at
 27 Patos Lagoon salt marshes, located at the south of the Neotropical region.
- 3. We sampled crane-flies from three points along the estuary by installing two
 Malaise traps at the low and high marsh strata of each point. Sampling was
 conducted uninterruptedly every fifteen days between August/2015 and
 July/2016, and collected insects were identified morphologically based on specific
 literature.
- 33 4. 5.248 crane-flies were identified in fourteen species and seventeen morphospecies. Frequency of occurrence of species revealed a gap in the presence 34 of constant species at the middle estuary. Dicranomyia, Gonomyia, Teucholabis 35 36 and Zelandotipula species were accessory only at the upper estuary, while Symplecta cana was accessory only at the lower estuary. This shows that different 37 species prefer distinct points along the estuary. Higher abundance of crane-flies 38 was correlated with elevated temperature and humidity. Symplecta pilipes was an 39 exception, presenting increase in abundance under lower temperatures. Seasonal 40 41 change in Tipuloidea species composition was observed, with higher evenness of Dicranomyia, Geranomyia, Rhipidia domestica and Symplecta cana (15-20%) 42 during summer, and dominance of Symplecta pilipes in winter (80%). 43

5. The gap at the middle estuary can possibly be due to stress caused by large
fluctuations in salinity at the area. In addition, the seasonal differences can have
significant ecological consequences such as modification of the Tipuloid species

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that compose the detritivorous and/or succivorous trophic guild, and consequently influence the upper trophic levels of the salt marsh environment.

49 Keywords: assemblage structure; crane-flies; diversity; seasonality; *Symplecta pilipes*.

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51 Introduction

Coastal ecosystems represent vast areas around the globe, and present singular 52 characteristics in terms of nutrient cycling, climate changes, geomorphological 53 characteristics and composition of fauna and flora (Lalli & Parsons 2006, Rezende et al. 54 2009). Among these environments, estuarine complexes are dynamic bodies of water with 55 56 a marine and freshwater interface created by freshwater discharge and intrusion of 57 saltwater from the ocean, leading to unique species composition and distribution. These communities require high physiological plasticity to tolerate the highly variable physical-58 chemical parameters (Williams & Williams 1998, Berezina 2003, Silberbush et al. 2005, 59 60 Chainho et al. 2006).

Salt marshes are common in estuarine ecosystems, and are known for their high 61 productivity. The water that permeates this vegetation carries organic matter into the 62 estuary/ocean, and has as important ecological role in sustaining aquatic food webs 63 (Boesch & Turner 1984, Huiskes 1988, Polis et al. 1997, Costa 1998). Spartina species 64 65 are the dominant halophyte vegetation in salt marshes, mainly of the lower stratum, and present a wide diversity of organisms associated with their roots and stalks (Curado et al. 66 2014) due to the abundant organic plant matter, creating a favorable niche for suspension 67 and deposit-feeders, as well as decomposers. Pennings & Bertness (2001) estimate that 68 around 10% of the carbon fixed in this environment flows through such organisms. The 69 aquatic plants and animals are influenced mainly by two factors: tide and/or salinity 70

71 oscillation. The multiple combinations by the levels of these factors, along with the 72 distance to estuarine or marine waters, creates a stratified mosaic of plant species known 73 as high and low marshes (Costa 1998). However, daily tides can vary geographically: for example, salt marshes along the Western coast of the United States are subjected to 200-74 75 300 cm oscillations (Pennings & Bertness 2001), while salt marshes along the Gulf of Mexico and the Patos Lagoon estuary (Brazil) only experience variations of 20-40 cm 76 (Stout 1984, Costa 1998). At these last regions, salinity is the main abiotic parameter that 77 78 influences how salt marshes are structured in terms of flora and fauna.

Insects are the most diverse and abundant group inhabiting salt marshes, with significant 79 80 ecological functions (Cheng 1976). For instance, ants and beetles have an important role 81 in the aeration of upper soil layers (Pétillon et al. 2008, Dummel et al. 2011, Bolico et al. 2012). Additionally, Insecta is one of the few groups capable of directly transferring 82 energy from the aquatic to the terrestrial environment, since many species present aquatic 83 84 larvae and winged-terrestrial adults (Keltz 1979, Polis et al. 1997). This is the case of flies and gnats, which are the dominating insects of salt marsh ecosystems (Cameron 85 1972, Kubátová-Hirsŏvá 2005). Despite the abundance and importance of Insecta for salt 86 marshes, little is known on the ecology, diversity and distribution patterns of insects at 87 these environments. 88

The abundance of insects is highly seasonal, with marked fluctuations at temperate zones where seasons are well defined (Wolda 1988, Silva *et al.* 2011, Kishimoto-Yamada & Itioka 2015). This is due to their direct relation with temperature, humidity, and precipitation (Gonçalves-Alvim & Fernandes 2001, Oliveira *et al.* 2006, Carrasco *et al.* 2014). Wind intensity is also an important variable since it carries winged insects to other

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areas or forces their landing, especially in the case of large species with wide wingspans
such as those of the Tipuloidea superfamily (3-36 mm; Gelhaus 2009).

96 Tipuloidea is the largest fly superfamily (Oosterbroek 2017), and its representatives are usually associated with humid ecosystems, such as the margins of bodies of water. Their 97 98 aquatic or semi-aquatic larvae occupy a wide range of habitats, while adults are found on adjacent vegetation (Gelhaus 2009), which makes salt marshes a potential nursery for this 99 family. The adult phase is relatively short when compared to the larval phase, many 100 101 species do not feed during this period, but instead use it for dispersion, nuptial flight and oviposition (Pritchard 1983). This short timeframe is advantageous for establishing the 102 103 influence of abiotic factors on crane-flies assemblages, and better understanding their 104 flight and reproduction. For example, it has been determined that most adult crane-flies present peak abundance in the summer and spring, when temperature and humidity are 105 high (Freeman 1968, Freeman & Adams 1972, Pritchard 1983); however, some species 106 107 can flourish during the winter, being capable of even tolerating freezing (Vanin & Masutti 2008, Taschereau et al. 2009, Hågvar et al. 2010). 108

Despite the importance of Tipuloidea to salt marshes and trophic webs, their distribution and ecology in these ecosystems is poorly understood, especially in the case of Limoniidae (Pritchard, 1983). In this manner, this study aims to better understand the diversity, distribution and seasonality of crane-flies at different zones and strata of salt marshes of the Patos Lagoon Estuary in South Brazil. Also, we determined their response to environmental parameters.

115 Materials and Methods

Study Area. The Patos Lagoon Estuary in South Brazil presents vast salt marshes,
dominated by few plant species: in the lower stratum (low marsh) by *Spartina alterniflora*

118 and Spartina densiflora; in the upper stratum (high marsh) by Scirpus maritimus, Scirpus olneyi and shrubs (including Myrsine parvifolia) (Costa et al. 2003). Three salt marsh 119 120 areas of the estuary were selected for sampling (Fig. 1). The first point was at Torotama Island located in the Upper Estuary (UE) (31° 55' 9.649" S; 52° 8' 25.300" W). This island 121 has around 40 km in extension, is covered almost exclusively by salt marshes, and has 122 the highest lacustrine influence. The second point was more central, located at Pólvora 123 Island in the Middle Estuary (ME) (32° 1' 19.538" S; 52° 6' 17.971" W). The marshes of 124 this point present a clear stratification between high and low strata. The third point, closest 125 to the estuary mouth and with the highest marine influence, was at the Barra Villa, Low 126 127 Estuary (LE) (32° 9' 13.028" S; 52° 6' 21.629" W). The low tide oscillation, large seasonal 128 variation in precipitation (Costa 1998) lead to irregular flooding of these salt marshes, configuring hypersaline conditions during summer and oligo-mesohaline conditions 129 throughout winter (D'Incao 1992). 130



Figure 1. Sampling areas at salt marshes of the Patos Lagoon Estuary, in South Brazil.

131 Sampling. Tipuloidea were sampled by installing two Malaise traps (one in each vegetation stratum) at each sampling point, close to the dominant vegetation, in a total of six traps. These traps function by intercepting flight, and are ideal for Tipuloidea due to

134 their lack of selectivity, leading to a representative capture of species with high reliability for seasonality studies (Kishimoto-Yamata & Itioka 2015). Sampling was conducted each 135 136 fifteen days over twelve months uninterruptedly, from August 2015 to July 2016. Some samples were lost due to winds and storms, leading to a total of 107 samples (38 UE, 38 137 138 ME, 31 LE). Collection cups containing 70% alcohol were changed every fifteen days, and collected insects were taken to the laboratory for morphological identification using 139 a magnifier and stereoscopic microscope. Insects were then stored in labeled tubes, and 140 after identification were deposited in the collection of the Rio Grande do Sul Zoobotanic 141 Foundation (Fundação Zoobotânica do Rio Grande do Sul - FZB/RS). Sampling was 142 143 conducted under license SISBIO # 50253-1.

Morphological identification. Genus identification was based on Alexander & Byers
(1981) and Gelhaus (2009), and species identification was based on Alexander (1912a,
1912b, 1913a, 1913b, 1927, 1935, 1937a, 1937b, 1938, 1945 and 1969), Andrew (2000),
and Starý & Brodo (2009).

Abiotic parameters. The following parameters were obtained from the National Institute of Meteorology (*Instituto Nacional de Meteorologia* – INMET, 2016): instant air temperature (°C), instant relative air humidity (%), wind speed (knots), and precipitation (mm/h). Salinity data was obtained from daily measurements along the estuary, conducted by the Decapod Crustacean Laboratory (*Laboratório de Crustáceos Decapodes*) of the Universidade Federal do Rio Grande's Oceanography Institute (*Instituto de Oceanografia – Universidade Federal do Rio Grande*).

155 Sampling efficiency. To evaluate sampling efficiency of Tipuloidea specimens, an 156 accumulation curve of the number of species was developed using random combinations 157 of the sampling units for the three sampling points. Mean and standard error was 158 calculated using the Mao-Tau method (Colwell *et al.* 2004). Additionally, we estimated
159 richness of species for each sampling point and for all the Patos Lagoon salt marshes,
160 using the CHAO method and a first order Jackknife, ideal estimators for abundance data
161 (Gotelli & Colwell 2011).

Frequency of occurrence. Species were classified according to their frequency of occurrence according to Dajoz (1978): constant (>50% of samplings), accessory (25-50%), accidental (<25%) and absent.

165 **Spatial and temporal variation.** Based on based on a Bray-Curtis dissimilarity matrix of species density (ind/15 days) a Principal Coordinates Analysis (PCoA) and a 166 167 Permutational Multivariate Analysis of Variance (PERMANOVA) was implemented to 168 evaluate differences in the assemblages between sampling points, marsh strata, and seasons (Anderson 2001, McArdle & Anderson 2001). This analysis was set to use 9999 169 permutations. The significant factors detected by PERMANOVA analysis were Pairwise-170 tested with Bonferroni correction. To compare species richness, equitability and 171 dominance between seasons, we constructed a Whittaker diagram (Melo 2008). To 172 evaluate the influence of abiotic parameters and seasons on species abundance, a 173 174 Canonical Correspondence Analysis (CCA) was done, followed by ANOVAs with 999 permutations to check the reliability of the analysis. Rare species (less than ten 175 individuals) were excluded from this analysis, and significance level was 5%. All 176 analyses were performed with software R, using Ape and Vegan packages. 177

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179 **Results**

180 **Sampled species.** A total of $5.248 (49.1 \pm 70.2 \text{ ind/collection cup})$ Tipuloidea individuals 181 were sampled, with an average 5.7 ± 3.8 species/collection cup. These were distributed

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over 31 species, of which fourteen were identified to the species level, and seventeen as morphospecies. Four belonged to family Tipulidae *sensu stricto*, and the remainder to Limoniidae. Morphological identification of one species was not possible, and it was therefore referred to as Limoniidae sp. *Dicranomyia* morphospecies were classified as a species complex due to their high morphological similarities, and is referred to as *Dicranomyia* complex. The list of species, along with total and relative abundance, and frequency of occurrence at the sampling points are shown in Table 1.

189	Table 1. Species list, total and relative abundance (RA), and frequency of occurrence (FO) of crane-flies at the sampled Patos
190	Lagoon Estuary salt marshes. Dark grey represents constant species; medium grey: accessory species; light grey: accidental
191	species; white: absent species.

Species	Abundance	RA	FO		
			Upper	Middle	Lower
Symplecta (Trimica) pilipes pilipes (Fabricius, 1787)	2674	0.51	0.79	0.79	0.84
Rhipidia domestica amazonensis Alexander, 1912	885	0.17	0.84	0.47	0.64
Dicranomyia complex	444	0.08	0.55	0.39	0.71
Geranomyia recisa Alexander, 1927	206	0.04	0.63	0.45	0.29
Ormosia sp1	199	0.04	0.53	0.45	0.55
Symplecta (Symplecta) cana (Walker, 1848)	143	0.03	0.26	0.13	0.45
Limoniidae sp.	119	0.02	0.55	0.11	0.10
Zelandotipula ringens (Alexander, 1935)	101	0.02	0.37		0.13
Ormosia sp2	79	0.02	0.50	0.08	0.13
Gonomyia (Paralipophleps) preuralis (Williston, 1896)	73	0.01	0.42	0.13	0.23
Polymera obscura Macquart, 1838	70	0.01	0.24	0.03	0.03
Gonomyia (Neolipophleps) condensa Alexander, 1938	53	0.01	0.42	0.16	0.10
Teucholabis (Teucholabis) fuscoapicalis	48	0.01	0.34		0.03
Molophilus sp.	33	0.01	0.24		
Dicranomyia (Dicranomyia) miseranda (Alexander, 1945)	29	0.01	0.31	0.13	0.13
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Polymera inornata Alexander, 1913	22	0.00	0.08	0.03	
Nephrotoma triobtusa Alexander, 1969	19	0.00	0.18		0.10
Toxorhina (Toxorhina) brasiliensis Alexander, 1913	13	0.00	0.21	0.03	0.03
Shannonomyia sp.	11	0.00	0.03		0.23
Geranomyia sp2	7	0.00	0.08	0.03	
Ozodicera (Ozodicera) cinereipennis Alexander 1937	4	0.00			0.13
Geranomyia sp3	4	0.00	0.05	0.05	
Dicranomyia sp3	2	0.00	0.03	0.03	
Dicranomyia sp4	2	0.00			0.06
Geranomyia sp5	2	0.00	0.03*		
Atarba sp	1	0.00			0.03*
<i>Tipula</i> sp	1	0.00	0.03*		
Geranomyia sp4	1	0.00	0.03*		
Gonomyia (Gonomyia) sp1	1	0.00			0.03*
Gonomyia (Gonomyia) sp2	1	0.00			0.03*
<i>Rhipidia</i> sp2	1	0.00	0.03*		

193 * Single specimen

Spatial variation. The total number of captured species was highest in the Upper estuary (26), followed by the Lower (22) and Middle estuary (17) (Table 2 and Fig. 2). The CHAO method estimated higher richness than the Jackknife analysis, but in both cases sampling efficiency was shown to be adequate, with the number of observed species falling within the confidence interval (Table 2).

The number of constant species at the extreme points of the estuary (upper and lower) 199 was respectively six and four. Symplecta pilipes was the only species constantly present 200 at all three points, and the only constant at the Middle estuary. From the lagoon to the 201 202 ocean, the number of accessory species was seven, four and two; the number of absent 203 species was four, 14, and nine. Dicranomyia miseranda, Limoniidae sp, Gonomyia 204 condensa, Ormosia sp2, Teucholabis fuscoapicalis and Zelandotipula ringens were accessory in the Upper estuary, and accidental in the other points, while Symplecta cana 205 was accessory in the Lower estuary and accidental in other two areas. Most of the 206 207 remaining species were classified as accidental for the three points, in the lagoon-to-ocean direction: 13, 13 and 16 species (Table 1). Molophilus sp., Ozodicera cinereipennis and 208 Dicranomyia sp4 were found to be exclusive to the Upper (Molophilus) and Lower 209 210 estuary (Ozodicera and Dicranomyia). The high salt marsh presented larger abundance than the low marsh for all points (PERMANOVA, F = 10.21, df = 1, p < 0.001; Fig. 5). 211



Figure 2. Estimated number of Tipuloidea species in different sampling sites of Patos Lagoon salt marshes. Peripheral-dotted lines indicate \pm 95% confidence interval.

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Table 2. Observed and estimated number of crane-fly species (± standard error) for each

sampling point of Patos Lagoon salt marshes, and in total.

	Upper	Middle	Lower	
N. of species	Estuary	Estuary	Estuary	Total
Observed	26	17	22	31
CHAO _{1 (SE)}	45.5 ± 23.0	29.2 ± 16.7	39.4 ± 22.9	43.1 ± 13.0
Jackknife1 (SE)	31.8 ± 3.09	21.9 ± 2.59	27.8 ± 3.38	37.9 ± 3.29
N. of samples	38	38	31	107

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Temporal variation. The tipulid assemblage did not vary significantly between seasons
in terms of abundance, with *Symplecta pilipes* representing 51% of the total crane-fly
abundance of the marshes. However, there was a clear difference in structure between

220 summer and winter, evidenced by the PCoA (Fig. 3) and PERMANOVA (F = 16.68, df = 3, p < 0.001). The Whittaker diagram also evidenced this difference (Fig. 4), with 221 summer presenting higher evenness between species (e.g. Dicranomyia complex, 222 223 Geranomyia recisa, Rhipidia domestica amazonensis and Symplecta cana; 15-20% of total abundance in this season. Meanwhile, in the winter there was a clear dominance 224 (80%) of Symplecta pilipes (Fig. 4). The spring was very similar to winter and, the autumn 225 was moderately similar to other seasons, presenting the highest number of species (Fig. 226 4). The fluctuation of abundance in the period was shown in Figure 5. 227



Figure 3. Principal Coordinate Analysis (PCoA) demonstrating structure in the Tipuloidea assemblage of Patos Lagoon salt marshes between the different seasons.



Figure 4. Whittaker diagram showing the evenness/dominance of crane-fly species of Patos Lagoon salt marshes between the different seasons

Influence of abiotic parameters. The abiotic variables among the sampling period are 228 shown in Table 3. The first two axis of the CCA used to evaluate the influence of abiotic 229 230 parameters on species abundance explained 87.67% of data variation (CCA1 F = 17.92, df = 1, p < 0.001; CCA2 F = 4.71, df = 1, p < 0.001; Fig. 6). Of the five used environmental 231 parameters, air temperature (F = 8.87, df = 1, p < 0.001) and relative air humidity (F = 232 233 7.01, df = 1, p = 0.03) significantly influenced the abundance of the crane-fly assemblage. Three types of correlations are noteworthy: 1) high correlation with relative humidity 234 235 displayed by the Group 1 presented by Dicranomyia complex, Polymera inornata, P. obscura, Molophilus sp. and Teucholabis fuscoapicalis; 2) positive correlation with 236 temperature, that includes species (Group 2) such as Geranomyia recisa, Symplecta cana, 237 Toxorhina brasilienses and Zelandotipula ringens; and 3) negative correlation with air 238 temperature and relative humidity, displayed exclusively by Symplecta pilipes. 239



Figure 5. Barplot of *Symplecta pilipes*, Group 1 (*Dicranomyia* complex, *Molophilus* sp., *Polymera inornata*, *P. obscura*, *Teucholabis fuscoapicalis* and *Rhipidia domestica amazonensis*) and Group 2 (other 23 Tipuloidea species) fluctuation among twelve months in each sampling point and high/low marshes. The lineplot presents the mean temperature in the same period.

Precipitation, salinity and wind did not influence Tipuloidea abundance (F = 1.49, df = 1, p = 0.53; F = 1.83, df = 1, p = 0.42, F = 2.65, p = 0.23).

Table 3. The mean of abiotic parameters for all sampling points and salinity mean for upper, middle and lower estuary among all seasons.

Mean	Temperature (°C)	Humidity (%)	Wind speed (knot)	Precipitation (mm/h)
Minimum	9.8	71.4	4.9	0.0000
Maximum	24.6	93.7	18.6	2.2000
Winter	14.7	83.7	11.8	0.4933
Spring	19.3	84.0	14.8	0.0111
Summer	23.6	83.3	13.3	0.0023
Autumn	14.7	86.9	10.5	0.0086
	Salinity Upper	Salinity Middle	Salinity Lower	
Minimum	0.0	0.0	0.0	
Maximum	10.9	10.8	14.0	
Winter	4.1	8.6	5.2	
Spring	3.1	10.9	0.7	
Summer	8.3	5.6	9.3	
Autumn	5.4	12.1	10.6	



Figure 6. Canonical Correspondence Analysis (CCA) of crane-fly species according to environmental parameters and seasons. Asterisks indicate significant influence.

Discussion

Tipuloidea occurrence. The salt marshes studied here displayed a very high abundance of crane-flies, with variation in composition and richness along the longitudinal estuarine gradient. Freshwater/marine transitional environments are considered stressful for Tipuloidea, since these insects are well adapted to less saline habitats. Tipuloidea larvae have been recorded at saline wetlands, but with a low number of species (zero to three) (Pupedis 1997, Giberson *et al.* 2001, MacKenzie 2005, Kratzer & Batzer 2007, MacKenzie *et al.* 2015). The high richness observed in the present study could be due to the sampling of adult specimens, which present a swarming behavior and are therefore easier to sample in the environment when compared to larvae. This is corroborated by the large number of species found in studies of adult crane-flies at other ecosystems such as boreal mire systems (29 species; Salmela & Ilmonen 2005) and eccentric bogs (23 species; Autio *et al.* 2013), which also present high decomposition rates of plant matter, allowing crane-flies to develop (Freeman 1967, Pritchard 1983, Petersen 2013).

Spatial variation of Tipuloidea. The frequency of occurrence of crane-flies along the studied salt marshes shows an interesting pattern: few species were considered constant at all points, likely due to the difficulty of Tipuloidea in occupying these highly dynamic environments (Yadamsuren *et al.*, 2015). A gap in occurrence was observed at the Middle estuary, possibly due to stress caused by large fluctuations in salinity at the area (Fernandes *et al.* 2005, Mont'Alverne *et al.* 2016), which is constantly influenced by both fresh and marine waters (Dijkstra *et al.*, 2014), and consequently hinder the allocation of species by physiological stress. Some species were classified as accessory only for the Upper estuary and accidental for other areas, which demonstrates that these species prefer areas with higher freshwater influence, likely due to terrestrial or freshwater origins

(Gathmann & Williams 2006). Meanwhile, *S. cana* showed the opposite pattern, preferring the Lower estuary's higher marine influence. These examples, along with *Molophilus* sp., *Ozodicera cinereipennis* and *Dicranomyia* sp4 – exclusive to the lower or higher estuary – show a preference for lacustrine or oceanic conditions along the estuary, which influences the determination of ecological niche and consequently the spatial distribution of these species (Giberson *et al.*, 2001). Crane-flies were more abundant in the high marsh in all sampling points (Fig. 5), which is expected considering that this stratum presents more shrubbery, allowing insects to land, copulate and feed, and providing shade (MacKenzie 2005, Marczak *et al.* 2013).

Temporal variation and influence of abiotic parameters. Tipuloidea species presented seasonal alternation with clear distinction in assemblage between summer (higher evenness between species) and winter (dominated by *Symplecta pilipes*). This was expected, since summer (with warmer temperatures) is known to favor reproduction and dispersal of most insect groups (Wolda 1988, Kishimoto-Yamata & Itioka 2015). For instance, in summer there is emergence of species belonging to genera *Geranomyia* and *Toxorhina*, which are important pollinizers that use their mouthparts to extract floral nectar (Alexander 1953). *Symplecta cana* also showed a unique peak in summer, which is also observed in the North hemisphere (Starý & Brodo 2009). However, some species such as *Molophilus* were not influenced by season or temperature, which could be explained by their temperature-independent and poikilothermic characteristics (Coulson *et al.* 1976). On the other hand, *Molophilus* is common at wetlands (Salmela & Ilmonen 2005), which explains its positive correlation with humidity. *Rhipidia domestica* was the commonest species in the autumn

Seasonal environmental changes lead to habitat alterations, allowing different species that depend on similar resources to occupy the same location but at different times (Chesson 2000). Our study showed the clear dominance of *Symplecta pilipes* at the Patos Lagoon salt marshes. This dominance was pronounced during winter, a generally hostile period for most Tipuloidea, which were observed more frequently in the summer. *S. pilipes* is currently the only cosmopolitan species of Tipuloidea (Driauach & Belqat 2016), and its high abundance and occurrence at all sampling points shows that this species presents ecological and physiological plasticity, tolerating unfavorable conditions such as low temperatures and low humidity values. This has likely led to its occupation of a wide range of habitats from coastal marshes, salt marshes and seacoasts (Starý 2014) to high altitude areas (Starý 2013). Other species of the Chioneinae subfamily (e.g. *Chionea* genus) also present this tolerance, occupying Arctic regions of the northern hemisphere (Vanin & Masutti 2008, Hågvar *et al.* 2010).

The parameters salinity and precipitation are highly determinant during larval phases due to the metabolic changes and/or emergence timing (Clark *et al.* 2004, Shaman & Day 2007, Chadsuthi *et al.* 2016). They were not significantly correlated with adults' abundance, however, as there is an influence on the first's stages of live, indirectly it may influence the adults. These parameters could become important during climatic events of larger temporal scale, such as El Niño/La Niña Southern Oscillations, which lead to prolonged periods of rain/drought in the extreme south of Brazil (Trenberth 1997, Garcia et al. 2004), and therefore cause significant structural changes in insect assemblages and communities (Carrasco *et al.* 2014, Kwok *et al.* 2016).

The observed seasonal differences in the crane-fly assemblage at salt marshes can have significant ecological consequences, including a constant change in Tipuloid species that

compose the detritivorous and/or succivorous trophic guild. These changes can influence the upper trophic levels of the salt marsh environment, such as insectivorous spiders, fish and birds (Polis *et al.* 1997, Britto & Bugoni 2015, Faria *et al.* 2016). Additionally, cranefly larvae are believed to play an important role in soil aeration and nutrient cycling, which could be affected by species changes (Yadamsuren *et al.* 2015). In this manner, the shift between species that compose the crane-fly assemblage of the Patos Lagoon salt marshes can implicate in differences in the ecosystem services these insects provide, such as decomposition of organic matter and transfer of energy between the aquatic and terrestrial environments.

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