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DIVERSIDADE DE PARASITOIDES (HYMENOPTERA) EM ÁREAS ORIZÍCOLAS
COM MANEJO ORGÂNICO E CONVENCIONAL E ANÁLISE FILOGENÉTICA DE
POPULAÇÕES DE *Telenomus podisi*

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DIVERSIDADE DE PARASITOIDES (HYMENOPTERA) EM ÁREAS ORIZÍCOLAS COM MANEJO ORGÂNICO E CONVENCIONAL E ANÁLISE FILOGENÉTICA DE POPULAÇÕES DE *Telenomus podisi*¹

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RESUMO

Parasitoides são importantes agentes de controle biológico em agroecossistemas e sua diversidade pode aumentar com a heterogeneidade do habitat. O conhecimento sobre as espécies associadas em cada sistema é importante para entender sua dinâmica e ajudar no manejo de diversas culturas, entre elas o arroz, que incluem ambientes naturais e com interferência antrópica. Espécies de Scelioninae, Telenominae e Teleasinae (Hymenoptera: Platygasteridae) destacam-se no controle biológico de várias pragas agrícolas, especialmente *Telenomus podisi*, embora haja alguma discordância sobre a monofilia do grupo. Assim, esta pesquisa tem dois objetivos. Primeiro, avaliar a contribuição da presença de vegetação natural próxima às áreas de cultivo do arroz e a influência de diferentes manejos da cultura (orgânica e convencional) na diversidade de himenópteros parasitoides e dentro das três subfamílias de Platygasteridae através dos gradientes de distância e nos estágios fenológicos da cultura. Segundo, através do uso dos marcadores moleculares COI e ITS2, analisar as relações entre populações de *T. podisi* de diferentes regiões biogeográficas. Para o primeiro objetivo, o trabalho foi realizado em duas áreas de arroz, uma com manejo orgânico (MO) e outra com convencional (MC), em Nova Santa Rita, RS, durante as safras 2013/2014 e 2014/2015. As coletas foram quinzenais durante o ciclo do arroz com armadilhas Malaise dispostas a diferentes distâncias em relação à vegetação nativa próxima da cultura do arroz. Para o segundo objetivo, 149 exemplares de *T. podisi* provenientes de 18 localidades em sete países foram amostrados, extraídos um fragmento de COI e todo ITS2 e amplificados através de primers específicos. Na primeira safra, foram 1104 indivíduos de parasitoides no MO (21 famílias) e 860 no MC (18 famílias). Na segunda safra, foram 1064 parasitoides no MO (19 famílias) e 389 no MC (16 famílias). Houve uma correlação negativa entre a distância da vegetação nativa e a abundância de parasitoides em áreas de MC. Os estágios fenológicos do arroz afetaram a composição de parasitoides no local. Dentro das três subfamílias, o total de indivíduos na primeira safra foram 268 no MO (31 morfoespécies) e 172 no MC (24 morfoespécies). Na segunda safra foram 151 indivíduos no MO (34 morfoespécies) e 93 no MC (21 morfoespécies). Os gêneros mais abundantes foram *Idris*, *Telenomus* e *Baeus*. *Telenomus podisi*, *Telenomus* sp.3, *Telenomus* sp.2 e *Telenomus* sp.1, foram as morfoespécies mais abundantes, respectivamente. Foram identificados 73 haplótipos de COI e ITS2. Mais de 70% dos haplótipos foram singletons e 89% foram exclusivos de uma área geográfica. Alguns haplótipos parecem estar evoluindo através de caminhos diferentes. Outros fatores podem estar exercendo pressão sobre esses haplótipos, como barreiras climáticas, deslocamento do hospedeiro e da flora associada.

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PARASITOIDS DIVERSITY (HYMENOPTERA) IN RICE FIELDS WITH ORGANIC
AND CONVENTIONAL MANAGEMENT AND PHYLOGENETIC ANALYSIS OF
Telenomus podisi POPULATION¹

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ABSTRACT

Hymenopteran parasitoids are important biological control agents in agroecosystems, and their diversity can be increased with habitat heterogeneity. Knowledge about associate's species in every system is important to understand the dynamic and to help the management of several crops, between them the rice crop, which include natural and anthropic environmental. Species of Scelioninae, Telenominae and Teleasinae (Hymenoptera: Platygasteridae) stand out in the biological control of several crop pests, especially *Telenomus podisi* although there is some disagreement about monophyly in *podisi* group. Thus, this research has two objectives. First, it was to evaluate the contribution of the presence of natural vegetation near rice-growing areas and the influence of different management of the crop (organic and conventional) on the diversity of parasitoids and within the three subfamilies of Platygasteridae (richness), through distance gradients, and on the phenological stages of the crop. Second, it was to use population genetic methods in conjunction with two molecular markers: COI and ITS2, to address relationships between *T. podisi* specimens from different biogeography regions. For the first aim, the work took place in two rice crops, one with organic management (O.M.) and another one with conventional (C.M.), in Nova Santa Rita, RS, Brazil, during the 2013/2014 and 2014/2015 seasons. The parasitoids were collected twice a month in the crop cycle with Malaise trap at different distances in relation to the native vegetation surrounding the rice crop. For the second aim, one hundred and forty-nine specimens of *T. podisi* were sampled from 18 localities in seven countries and a fragment extraction of COI and the entire ITS2 region was amplified through specific primers. In the first season, 1104 individuals of parasitoids were sampled in O.M. (21 families) and 860 in C.M. (18 families). In the second season, 1064 parasitoids in O.M. (19 families) and 389 in C.M. (16 families) were sampled. There was a negative correlation between distance from native vegetation and parasitoid abundance in CM areas. The phenological stages of rice affect the parasitoid assemblage on site. Within the three subfamilies, 268 individuals were sampled in the first season in O.M. (31 morphospecies) and 172 in C.M. (24 morphospecies). In the second season, 151 individuals in O.M. (34 morphospecies) and 93 in C.M. (21 morphospecies) were sampled. The most abundant genera were *Idris*, *Telenomus* and *Baeus*. *Telenomus podisi*, *Telenomus* sp.3, *Telenomus* sp.2 and *Telenomus* sp.1, were the most abundant morphospecies, respectively. Seventy-three COI and ITS2 haplotypes were identified. More than 70% of haplotypes were singletons and 89% unique to a geographic area. Some haplotypes seem to be evolving through different pathways. Others features may be putting pressure on these haplotypes, such as climatic barriers, shift of host and flora associate.

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1 INTRODUÇÃO

A maioria dos organismos considerados pragas na cultura do arroz tem registro de ocorrência de parasitismo por himenópteros parasitoides (Islam *et al.*, 2012). O estudo da taxonomia, diversidade e ecologia populacional desses inimigos naturais proporciona subsídios para um maior conhecimento desse grupo e para sua utilização eficaz no controle das pragas.

O controle biológico se baseia em um processo ecológico natural da rede alimentar em que os parasitoides são animais topo de cadeia e, conseqüentemente, controladores das populações de hospedeiros que, frequentemente, são insetos fitófagos (Pywell *et al.*, 2011).

Áreas de vegetação nativa preservadas dentro de propriedades rurais contribuem para a conservação de processos ecológicos e da biodiversidade do local ou, ao menos, minimizam as perdas (Altieri *et al.*, 2003). Estudos sobre comunidades de insetos, como levantamentos de diversidade taxonômica e funcional e a relação com a paisagem agrícola, possibilitam o entendimento dos papéis destes organismos no agroecossistema. Tendo como base este conhecimento, pode-se trabalhar na manutenção de áreas que sirvam de reservatório de inimigos naturais que possam atuar no controle biológico de pragas existentes nas culturas, visto que são um meio para se construírem alternativas sustentáveis em agroecossistemas.

As práticas relacionadas aos conceitos da agricultura sustentável e da biologia da conservação visam manter a produtividade dos ecossistemas, preservando a biodiversidade e

limitando as práticas ambientalmente destrutivas. Estes conceitos partem do princípio de que os ecossistemas agrícolas e os naturais são geridos por processos ecológicos semelhantes e que os mesmos fatores determinam sua sustentabilidade (Altieri *et al.*, 2003).

Entretanto, os sistemas agrícolas de modo geral são simplificados, constituindo-se de grandes extensões de monoculturas com reduzida diversidade biológica (Jonsson *et al.*, 2015). A diversificação do ambiente agrícola possibilita um aumento das comunidades de organismos associados a este, através da implementação de arquiteturas vegetais que suportem populações de inimigos naturais ou que causem efeito direto sobre fitófagos pragas (Gliessman, 2001). Em algumas culturas, por outro lado, isso não se torna viável, como é o caso do arroz irrigado que exige uma área inundável que possa ser, em algumas ocasiões, drenada, dificultando a associação com outras espécies vegetais, além de outras constantes intervenções de manejo. Sistemas agrícolas deste tipo têm evoluído para habitats que são muito mais simples em estrutura e mais pobres em espécies que os outros. Assim, a implantação ou conservação de uma vegetação diversificada no entorno, ou próxima da cultura, torna-se uma alternativa para a manutenção da diversidade desses agroecossistemas. Além disso, o conhecimento da variação na abundância e composição de diferentes grupos de parasitoides em relação à cultura serve como base para o desenvolvimento de planos de manejo adequados para diminuição de pragas específicas.

Platygastridae é uma família de himenópteros parasitoides que utiliza como hospedeiros principalmente os pentatomídeos. Esses hemípteros incluem espécies registradas como pragas da cultura do arroz (SOSBAI, 2014). Os platigastrídeos ovipositam nos ovos do hospedeiro, atacando-os no início de seu desenvolvimento. Possuem alta especificidade em relação aos mesmos (Hanson & Gauld, 2006), sendo estas características importantes para um controle biológico eficaz (Van Driesche & Bellows, 1996). Entretanto, para o sucesso no controle

biológico, é necessária a correta identificação da espécie objeto de trabalho. As potenciais consequências de uma taxonomia falha conduz a cascatas de erros que afetam hipóteses e ideias científicas, sendo comumente subestimadas ou ignoradas, mas podendo ter graves implicações ecológicas e econômicas (Wägele *et al.*, 2011). A taxonomia em nível específico dessas vespas parasitoides, requer trabalho exaustivo em função da alta similaridade morfológica. Mais recentemente, diversos trabalhos com himenópteros parasitoides têm recomendado a adição de técnicas moleculares para confirmação de variações intraespecíficas em espécies crípticas, ou seja, similares morfológicamente (Johnson, 2011; Derocles *et al.*, 2016).

Ferramentas de diagnóstico molecular proporcionam um meio rápido e preciso de identificação quando caracteres morfológicos não são suficientes. Em contraste com as características morfológicas, dados moleculares são expressões de modificações em nível de gene e são menos influenciados pelo meio.

Os objetivos do trabalho foram avaliar a contribuição da presença de fragmentos de vegetação natural através de gradientes de distância sobre a riqueza e abundância de himenópteros parasitoides em sistemas orizícolas com diferentes manejos da cultura e sob os diferentes estágios de desenvolvimento da planta e analisar as variações de populações de *Telenomus podisi* (Ashmead) (Hymenoptera: Platygasteridae) através de dois marcadores moleculares.

2 REVISÃO BIBLIOGRÁFICA

2.1 A cultura orizícola

O arroz (*Oryza sativa* L.) é uma gramínea anual originária da Ásia e sua domesticação ocorreu há cerca de 10 mil anos (Islam *et al.*, 2012). Foi trazido pelos colonizadores portugueses ao Brasil, tendo os espanhóis o introduzido na América Central e em partes da América do Sul (Pereira *et al.*, 1999). No Rio Grande do Sul (RS), acredita-se que, em meados do século XVIII, os açorianos já cultivavam o arroz na região dos Sete Povos das Missões (Pereira *et al.*, 1999; Zilli & Barcellos, 2006).

As áreas próprias para o cultivo do arroz irrigado caracterizam-se por apresentar uma topografia plana e de difícil drenagem, onde os solos permanecem saturados em períodos de maior precipitação pluvial. Essas características, normalmente desfavoráveis para outras culturas, tornam-se adequadas para o cultivo do arroz, facilitando a manutenção de uma lâmina d'água sobre a superfície e dificultando a lixiviação de nutrientes (SOSBAI, 2014). Para um aproveitamento eficiente desses solos, é necessário um processo de sistematização, de modo que se adotam quadros fixos, regulares e, em geral, de pequenas dimensões, separados por taipas permanentes (EMBRAPA CLIMA TEMPERADO, 2005). As taipas são pequenos taludes de terra delimitando quadros com irrigação de água, tendo altura variável de acordo com o nível de água dentro da lavoura (Brouwer, 1988). Em algumas situações de topografia, é viável utilizar as áreas entre taipas em curvas de nível (EMBRAPA CLIMA TEMPERADO, 2005).

Para uma melhor comunicação entre técnicos e produtores e a adoção de tecnologias e práticas de manejo eficientes no desenvolvimento do cultivo do arroz, faz-se necessário o uso de uma escala apropriada para expressar o desenvolvimento da planta (SOSBAI, 2014). De acordo com Counce *et al.* (2000), o ciclo de desenvolvimento do arroz pode ser dividido em três fases principais, a saber: plântula, vegetativa e reprodutiva. A duração de cada fase varia em função do cultivar, época da semeadura, região de cultivo e condições de fertilidade do solo, por exemplo. Para a maioria das cultivares utilizadas no Rio Grande do Sul, as quais necessitam temperatura ao redor de 24 a 30 °C e radiação solar elevada, o ciclo é entre 100 e 140 dias (EMBRAPA CLIMA TEMPERADO, 2005).

O Brasil apresentou uma produção anual entre 11 e 13 milhões de toneladas de arroz nas últimas safras (IBGE, 2016), sendo responsável por cerca de 79,3% da produção do Mercosul (SOSBAI, 2014). O Rio Grande do Sul se destaca, sendo responsável por cerca de 69% do total produzido no Brasil, com uma produção de mais 8,6 milhões de toneladas em 2016 e um rendimento médio de 7.700 kg/ha (CONAB, 2016).

Quanto ao aspecto social, a importância do arroz é representada pela possibilidade de ser cultivado tanto em pequenas como em médias e grandes áreas. Esta flexibilidade da cultura permite que a agricultura familiar e a empresarial se desenvolvam e utilizem o arroz como alternativa para geração de renda e de empregos (SOSBAI, 2014). No último Censo Agropecuário, foram registrados para o RS 354.677 estabelecimentos de agricultura familiar que produziram 3,2 ton de arroz (base casca) em uma área colhida de 1.167 ha (IBGE, 2006). Atualmente, este número deve ter crescido, tendo em vista o aumento registrado de produção geral (IBGE, 2016).

2.2 Manejo de pragas em agroecossistemas

As atividades agrícolas têm contribuído muito para redução dos recursos naturais e da qualidade ambiental no Brasil, tanto na Região Centro-Oeste, Oeste da Bahia e na Região Sul (Barrigossi *et al.*, 2004). Com a modernização da agricultura, a produção em grandes áreas de monocultura passou a predominar, em detrimento dos princípios ecológicos e da preocupação com o ambiente (Altieri *et al.*, 2003). Os cultivos em grande escala promovem a simplificação da biodiversidade e a formação de um sistema artificial, que requer constante intervenção humana com utilização de agroquímicos que, apesar de contribuir para elevar a produtividade temporariamente, apresenta diversas desvantagens sob o ponto de vista ambiental e econômico (Barrigossi *et al.*, 2004).

As áreas cultivadas de arroz são circundadas por habitats aquáticos e terrestres, compreendendo um mosaico de ambientes em transformação. Comparados com outras culturas agrícolas, estes podem abrigar grande diversidade biológica, mantida pela rápida colonização, assim como pela rápida reprodução e crescimento dos organismos (Fritz, 2009). A fauna associada a estes sistemas compreende vertebrados e invertebrados que habitam a vegetação, a água e o solo dos campos orizícolas (Hook, 1994).

O manejo de pragas e doenças é um dos principais pontos que deve ser considerado em relação à competitividade e sustentabilidade da agricultura (Altieri *et al.*, 2003). Este tem sido focado mais no aspecto econômico que no ambiental (Waquil *et al.*, 2006). O ideal seria a escolha de um método a ser empregado pautado em avaliações socioeconômica e ambiental, utilizando-se da associação de várias técnicas como resistência varietal, controle biológico, químico, cultural, dentre outros (Barrigossi *et al.*, 2004). Segundo os mesmos autores, pesquisadores concordam que a agricultura moderna tem confrontado muitos dos princípios ecológicos básicos e que as perdas de produção devido às pragas ainda são elevadas, apesar do

consumo de agrotóxicos ter aumentado em escala mundial. Uma alternativa tem sido resgatar os princípios ecológicos na forma de produzir. Assim, além da inovação tecnológica, deve ser considerada a complexidade das interações entre os recursos, pessoas e o ambiente (Rodrigues *et al.*, 2003).

A compreensão de como as práticas de manejo agrícola interferem negativamente na biodiversidade dos ecossistemas agrícolas, neste caso, da cultura do arroz, permitirá aos produtores a incorporação de estratégias de conservação das espécies e restauração de áreas degradadas (Fritz *et al.*, 2008).

Na busca de práticas agrícolas com menos impactos ambientais, surgem alternativas orientadas pelo conhecimento dos processos ecológicos que ocorrem em agroecossistemas (Gliessman, 2001). No Brasil, a Lei 10.831/2003 e o Decreto 6.323/2007 (BRASIL, 2003; BRASIL, 2007) deram início à regulamentação da agricultura orgânica. Nesses instrumentos, o termo institucionalizado foi o “orgânico”, que engloba todos os outros: biodinâmico, natural, biológico, agroecológico e permacultura.

A produção orgânica de arroz irrigado está baseada na eliminação do uso de insumos químicos sintéticos, como fertilizantes, agrotóxicos e reguladores de crescimento, sendo privilegiadas tecnologias alternativas como mecanização, preparo do solo, adubação e controle de pragas, incluindo plantas daninhas, doenças e insetos (EMBRAPA CLIMA TEMPERADO, 2009). Neste sistema, a diversidade na produção vegetal é assegurada, no mínimo, pela prática de associação de culturas a partir das técnicas de rotação e consórcios (EMBRAPA CLIMA TEMPERADO, 2009). Além disso, a manutenção das áreas de preservação permanente e a atenuação da pressão antrópica sobre os ecossistemas naturais e modificados são aspectos ambientais que os sistemas de produção orgânica de arroz devem buscar para obtenção da certificação (EMBRAPA CLIMA TEMPERADO, 2009).

2.3 Pragas associadas ao arroz

Algumas espécies de insetos e outros fitófagos que ocorrem na cultura do arroz irrigado podem atingir níveis populacionais de dano econômico e causar perdas de produtividade da ordem de 10 a 35 % (Martins *et al.*, 2009).

De maneira geral, a planta de arroz pode sofrer danos em diferentes partes por diversos grupos de fitófagos (EMBRAPA CLIMA TEMPERADO, 2005). As sementes, plântulas e raízes são atacadas, principalmente, por larvas e adultos de coleópteros, por pássaros e moluscos (SOSBAI, 2014). Os colmos e folhas são alvos de insetos mastigadores, sugadores e raspadores, sendo os dois primeiros grupos os mais importantes. Os grãos, por sua vez, sofrem ataques de um conjunto de insetos sugadores que afetam diretamente a quantidade e a qualidade do arroz (SOSBAI, 2014).

As pragas da orizicultura podem ser divididas em primárias e secundárias, causando danos diretos ou indiretos (Domiciano, 2001). Entre as consideradas primárias, estão os lepidópteros da família Noctuidae, como as lagartas-dos-milharais e capinzais (*Pseudaletia sequax* Franclemonte e *Pseudaletia adultera* Schaus), a lagarta-militar [*Spodoptera frugiperda* (Smith)], coleópteros Curculionidae, como a bicheira-da-raiz-do-arroz [*Oryzophagus oryzae* (Costa Lima)], e os hemípteros Pentatomidae, o percevejo-do-colmo (*Tibraca limbativentris* Stål) e o percevejo-do-grão-do-arroz [*Oebalus poecilus* (Dallas)]. As pragas de importância secundária são os coleópteros pulga-do-arroz (*Chaetocnema* sp., Chrysomelidae) e cascudo-preto (*Euetheola humilis* Burmeister, Scarabaeidae), os hemípteros pulgão-da-raiz [*Rhopalosiphum rufiabdominalis* (Sasaki), Aphididae] e o percevejo-do-capim [*Collaria scenica* (Stål), Miridae], os lepidópteros broca-da-cana [*Diatraea saccharalis* (Fabricius), Pyralidae], lagarta-boiadeira (*Nymphula indomitalis* Berg, Pyralidae) e o coleóptero broca-do-colo [*Ochetina uniformis* (Pascoe), Curculionidae] (Gomes & Magalhães-Junior, 2004).

Embora haja registros de muitas espécies de insetos danificando o arroz, a maioria destas pode apresentar outras funções no agroecossistema orizícola. Settle *et al.* (1996) em levantamento realizado em cultura de arroz irrigado na Indonésia, por exemplo, registraram a maioria dos indivíduos como inimigos naturais de pragas, sendo 40% predadores, 24% parasitoides, 19% detritívoros e somente 17% fitófagos.

2.4 Controle de insetos na cultura do arroz

Para o controle de fitófagos, o manejo convencional utiliza principalmente inseticidas dos grupos químicos neonicotinoide, diamida, piretroide e carbamato (AGROFIT, 2016), cujos sítios de ação primária são agonistas de receptores nicotínicos da acetilcolina, moduladores de receptores de rianodina, moduladores de canais de sódio e inibidores de acetilcolinesterase (Salazar Cavero, 1998). Esses inseticidas, portanto, são classificados como neurotóxicos e não seletivos, podendo atingir tanto fitófagos como inimigos naturais e polinizadores (Omoto & Guedes, 2000).

Por outro lado, as recomendações técnicas para o controle de insetos e outros fitófagos no arroz irrigado no Rio Grande do Sul preconizam o emprego de medidas integradas de manejo que reduzam os danos causados à cultura (SOSBAI, 2014).

Em sistemas orgânicos, como o do arroz, a restrição ao uso de inseticidas é ainda maior do que no Manejo Integrado de Pragas (MIP), no qual é previsto um sistema de manejo sustentável da unidade de produção com enfoque sistêmico que privilegia a preservação ambiental, a agrobiodiversidade, os ciclos biogeoquímicos e a qualidade de vida humana (EMBRAPA AGROBIOLOGIA, 2004).

O controle biológico conservativo, uma das importantes técnicas de manejo, atua com outros fatores do meio e contribui decisivamente para diminuir a importância dos fitófagos do

arroz, devendo os inimigos naturais serem preservados pela utilização adequada das práticas de manejo (Fritz *et al.*, 2008).

2.5 Importância dos fragmentos vegetais naturais próximos às áreas de cultivo

A intensificação da agricultura frequentemente prejudica os serviços ecológicos que poderiam beneficiar a produção (Jonsson *et al.*, 2015), como por exemplo a preparação do solo e aplicação de pesticidas, além de outros fatores (Tscharntke *et al.*, 2005). O aumento da perturbação pode ser mais prejudicial aos inimigos naturais do que para os insetos pragas, podendo enfraquecer a supressão dos mesmos (Krauss *et al.*, 2011; Jonsson *et al.*, 2012), resultando em uma necessidade ainda maior de agroquímicos para resolver os problemas locais com os ataques aos cultivos (Jonsson *et al.*, 2015). Além disso, o aumento dos campos de cultivo, contínuos em extensão e dominados por poucas espécies vegetais, leva a homogeneização do local, tornando-os simples em estrutura e composição de espécies (Margosian *et al.*, 2009). Essa simplificação aumenta a concentração de recursos que estão disponíveis para as pragas (Margosian *et al.*, 2009; Tscharntke *et al.*, 2012) e reduz os habitats não agriculturáveis que suportam os inimigos naturais (Chaplin-Kramer *et al.*, 2011).

Buscando entender e organizar as interações ecológicas, surge a abordagem do sistema “bottom-up”. Em uma comunidade, este sistema prediz que o equilíbrio ecológico é ditado de baixo para cima, ou seja, em que uma comunidade de vegetação mais diversa é associada com alta abundância e riqueza de predadores e parasitoides que se alimentariam de herbívoros em épocas específicas, realizando um controle biológico destes (Root, 1973; Haddad *et al.*, 2001; Pywell *et al.*, 2011; Nemeč *et al.*, 2014). Em um agroecossistema o controle “bottom-up” se refere a quanto um predador (ou grupo de predadores ou parasitoides) influencia a estrutura ou a dinâmica populacional do ecossistema (Pywell *et al.*, 2011).

Para um uso efetivo no controle biológico conservativo é necessário saber se esse equilíbrio do sistema favorece um aumento nas taxas de parasitismo. Fenoglio *et al.* (2013) analisaram a taxa de parasitismo associado a áreas com diferentes gradientes de isolamento e a associação dos mesmos com o aumento da riqueza de parasitoides. Apesar de o trabalho ser conduzido em ambientes urbanos, o mesmo mostra como a fragmentação de habitats e as áreas em mosaico afetam a taxa de parasitismo e a relação dos mesmos com a riqueza de parasitoides, podendo ser usado como base para agroecossistemas. Neste trabalho os autores encontraram que a taxa de parasitismo foi maior com o aumento no tamanho da área e diminuiu com o aumento no grau de isolamento. Além disso, os efeitos, tanto no tamanho quanto na isolação do habitat na taxa de parasitismo, foram indiretos, mediados pelas mudanças na abundância dos hospedeiros e da riqueza de espécies parasitoides. Conforme os autores, a riqueza de parasitoides foi significativamente correlacionada às taxas de parasitismo, indicando que o número de espécies parasitoides, independentemente da abundância de hospedeiros, influenciou a proporção de larvas parasitadas.

As mudanças nas paisagens dos agroecossistemas podem alterar a composição vegetal assim como os recursos disponíveis e os habitats, modificando o acesso dos insetos a alimentação e refúgios, afetando assim, sua estabilidade populacional (Tschardtke *et al.*, 2012). A alta estabilidade de insetos poderia então ser benéfica para o controle biológico de pragas nos agroecossistemas (Haddad *et al.*, 2011). De acordo com Zaho *et al.* (2015), o alto nível de heterogeneidade de habitat em áreas de cultivo de trigo é um importante estabilizador da distribuição espacial de afídeos, parasitoides primários e secundários. Segundos os autores, a relação entre esta estabilidade e heterogeneidade de habitat pôde ser descrita por uma regressão linear, que indicou que os níveis tróficos mais altos (parasitoides) foram os mais sensíveis para as mudanças na vegetação da paisagem do que o nível trófico mais baixo (afídeos), levando a

conclusão de que o aumento na heterogeneidade de habitat pode afetar ambos, afídeos e parasitoides, mas com os últimos sendo mais sensíveis a esse aumento.

Letourneau *et al.* (2012) analisaram a riqueza e abundância de parasitoides da família Tachinidae (Diptera) em cultivos vegetais (brócolis, couve, cenoura, repolho, alface, entre outras) com áreas adjacentes variando desde complexos mosaicos de vegetação até áreas homogêneas de produção agrícola na Califórnia. Os autores constataram associação positiva da riqueza e abundância de Tachinidae com áreas permanentes de vegetação e a negativa relação com áreas de cultivo o que suporta a conclusão de que a efetiva conservação de parasitoides nativos aconteceu pela preservação e/ou restauração de um habitat com estágio sucessional avançado dentro da paisagem agrícola. Esta conclusão também foi feita por Marino *et al.* (2006), estudando os parasitoides de lepidópteros pragas de diferentes culturas na América do Norte. Segundo os autores, os lepidópteros analisados eram atacados principalmente por parasitoides que tinham hospedeiros alternativos que se alimentam exclusivamente em vegetação com estágio sucessional avançado.

A discussão a respeito do uso de controle biológico conservativo ser aplicável somente em pequenas áreas tem sido discutida por pesquisadores como Merck & Pereira (2015), que defendem a remodelação da agricultura em larga escala, buscando a produtividade e, ao mesmo tempo, tornando-a integrada ao sistema natural. Assim, o controle biológico através da conservação, restauração ou estabelecimento de vegetação nos cultivos ou ao redor deles apresenta-se como uma estratégia promissora (Thies *et al.*, 2011).

2.6 Parasitoides: inimigos naturais de pragas agrícolas

Os inimigos naturais que desempenham um papel fundamental no manejo das culturas incluem predadores, parasitoides e microrganismos patogênicos capazes de reduzir a densidade populacional das pragas e, conseqüentemente, o dano das mesmas (Heinrichs & Barrion, 2004).

Os parasitoides são insetos cujas larvas alimentam-se de outros artrópodes, geralmente insetos, levando à morte o hospedeiro (Godfray, 1994). Os adultos são de vida livre, mas ovipositam próximo, sobre ou no interior de outros insetos, em aranhas ou isópodes, com a larva se desenvolvendo no interior ou na superfície do hospedeiro (Jervis & Kidd, 1991). No início do seu desenvolvimento, a larva causa um dano pouco aparente ao hospedeiro, mas acaba consumindo-o quase totalmente e, portanto, matando-o (Godfray, 1994). Quando está totalmente desenvolvido, o parasitoide emerge de onde aparentemente deveria emergir seu hospedeiro. Geralmente, apenas um parasitoide se desenvolve em cada hospedeiro, mas, muitas vezes, vários indivíduos podem compartilhar o mesmo (Begon *et al.*, 2007). Segundo os mesmos autores, a grande maioria dos insetos é atacada por, pelo menos, uma espécie de parasitoide e este, por sua vez, pode ser atacado por outros.

Os parasitoides constituem um dos grupos de agentes de controle biológico mais importante no controle de populações de pragas de sistemas agrícolas, tanto pela ocorrência natural das espécies no ambiente, quanto pela sua utilização em programas de controle biológico aplicado (Hanson & Gauld, 2006). Sua diversidade, associada a diferentes sistemas de cultivos, é determinada por fatores ambientais, biológicos e de manejo (Chay-Hernandez *et al.*, 2006). Em grandes monoculturas, a diversidade de parasitoides pode ser suprimida por pesticidas, simplificação de vegetação e outros distúrbios ambientais (Altieri, 2002). Em agroecossistemas menos perturbados, a diversidade mostra-se relacionada à diversidade de cultivos, plantas invasoras, cobertura do solo e vegetação nativa próxima aos cultivos (Onody, 2009; Liere *et al.*,

2015). A ocorrência natural dos parasitoides nos agroecossistemas é um fator de grande importância para a redução da infestação de pragas, portanto, o conhecimento desta fauna e a conservação do meio ambiente tornam-se imprescindíveis para o sucesso de sua conservação nos locais em que ocorre (Hanson & Gauld, 2006).

As ordens Hymenoptera e Diptera são as que apresentam maior representatividade dentre os grupos de parasitoides (Gullan & Cranston, 2008). Em relação aos himenópteros parasitoides, vários são os trabalhos que afirmam a importância destes como reguladores naturais das populações de vários hospedeiros, o que os torna essenciais para a manutenção do equilíbrio ecológico (Thies *et al.*, 2011; Purnomo & Wright, 2016). Esses organismos são abundantes na natureza e ocupam os mais diversos tipos de ambientes disponíveis (Gallo *et al.*, 2002). Para a ordem Hymenoptera, um total de 153.088 espécies existentes foram descritas, além de 2.429 espécies extintas, sendo considerada uma das mais ricas, atrás apenas de Coleoptera e Lepidoptera (Aguiar *et al.*, 2013).

No MIP, os parasitoides de ovos são considerados, em vários países, como os principais inimigos naturais de Pentatomidae (Pacheco & Corrêa-Ferreira, 2000). Além disso, existem várias espécies de himenópteros parasitoides importantes no controle de ovos e lagartas de Lepidoptera (Gondim *et al.*, 2011; Gates *et al.*, 2012; Luna *et al.*, 2015).

Vários são os estudos relacionando parasitoides a diferentes pragas da cultura do arroz. Em um trabalho realizado no Maranhão, foi registrado um parasitismo de 23,4% em ovos de *T. limbativentris*. As espécies encontradas foram *Telenomus podisi* Ashmead, *Trissolcus urichi* (Crawford) (Hymenoptera: Platygasteridae) e *Ooencyrtus submetallicus* (Howard) (Hymenoptera: Encyrtidae) (Maciel *et al.*, 2007). Em lavouras arrozeiras, em Santa Catarina, as mesmas espécies foram encontradas em ovos do percevejo-do-colmo, apresentando, entretanto, um índice superior a 70% (Riffel, 2007). Em lavouras do Rio Grande do Sul, no município de

Eldorado do Sul, *T. podisi* teve um índice de parasitismo de 75% sobre *T. limbativentris* (Idalgo *et al.*, 2013). Ainda no RS, em lavouras de manejo orgânico em Águas Claras, Viamão, os parasitoides mais frequentes em lavouras de arroz foram os das famílias Mymaridae e Platygastriidae (Simões-Pires *et al.*, 2016).

São relacionadas também 17 espécies de himenópteros parasitoides, pertencentes às famílias Braconidae, Eulophidae, Ichneumonidae, Trichogrammatidae, parasitando *S. frugiperda* na cultura do arroz (EMBRAPA ARROZ E FEIJÃO, 2008). Para *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), são citadas espécies de parasitoides de lagartas das famílias: Platygastriidae, Braconidae, Ichneumonidae, Chalcididae, Eulophidae e Perilampidae (EMBRAPA ARROZ E FEIJÃO, 2008). No que se refere à broca-do-colmo, *D. saccharalis*, os himenópteros parasitoides de ovos, *Telenomus* spp. e *Trichogramma* spp. são destaque e, para lagartas, o principal é *Cotesia flavipes* Cameron [= *Apanteles flavipes* (Cameron)] (Braconidae) (EMBRAPA ARROZ E FEIJÃO, 2008).

Em relação ao gorgulho-aquático *O. oryzae*, entretanto, não há referência de parasitoides. Os percevejos-das-panículas, *O. poecilus* e *Oebalus ypsilon* (De Geer) (Hemiptera: Pentatomidae) possuem como parasitoides de ovos *Trissolcus* (= *Microphanurus*) *mormideae* Lima e *Telenomus mormideae* Lima (Hymenoptera: Platygastriidae) (EMBRAPA ARROZ E FEIJÃO, 2008). Fritz *et al.* (2008) realizaram um levantamento bibliográfico a respeito de registros de ocorrência de insetos pragas e inimigos naturais em agrossistemas orizícolas irrigados no Brasil. Entretanto, embora existam estes registros, poucos são os trabalhos que avaliam as populações de inimigos naturais ou realizam análises de diversidade destes.

Através do estudo de similaridade na composição das famílias e espécies mais abundantes nas áreas preservadas próximas aos cultivos, infere-se que estes refúgios de

vegetação natural podem servir de repositório de inimigos naturais de insetos pragas das culturas (Bambaradeniya & Edirisinghe, 2008; Ferreira *et al.*, 2014; Da Silva *et al.*, 2016).

Apesar das evidências da importância dos fatores ambientais como condições climáticas, solo e feições fitogeográficas, afetando os parasitoides de pragas do arroz, o uso da manipulação do habitat para aumentar o controle biológico da segunda maior cultura de cereal do mundo (SOSBAI, 2014) é surpreendentemente sub-representada na literatura (Way & Javier, 2001; Gurr *et al.*, 2011), o que ressalta a relevância do trabalho aqui desenvolvido.

2.7 Importância da família Platygasteridae em culturas agrícolas

Dentro da família Platygasteridae, os integrantes das subfamílias Teleasinae, Scelioninae e Telenominae são parasitoides de ovos e possuem ampla gama de hospedeiros entre lepidópteros, hemípteros e dípteros (Hanson & Gauld, 2006). Segundo os mesmos autores, a maioria das espécies mostra alto grau de especificidade, algumas restritas somente a uma espécie hospedeira e, a maior parte, a uma só família (Hanson & Gauld, 2006).

A composição das famílias e espécies de parasitoides em diferentes locais e cultivos também pode ser alterada em decorrência das pragas que lhes servem de hospedeiros. A maior abundância de parasitoides, encontrados no trabalho de Bambaradeniya & Edirisinghe (2008) em arroz no Sri Lanka, por exemplo, foi da família Mymaridae, (39%), que atacam os insetos *Nilaparvata lugens* (Stål) e *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae), importantes pragas naquela região. Entretanto, Gurr *et al.*, (2011) alertaram que o fato de Platygasteridae não aparecer como uma família representativa parasitando Delphacidae na região, pode ser em razão da identificação errada ou escassez de pesquisa na região.

Trabalhos anteriores já mostram a importância de Platygastriidae em áreas orizícolas, atuando como controlador da abundância de pragas desta cultura (Fritz *et al.*, 2008 e 2011; Da Silva *et al.*, 2016).

Em outras regiões do mundo, como a Ásia e África, Platygastriidae também é uma família importante para a cultura do arroz. No continente asiático, *Platygaster oryzae* Cameron é um parasitoide do galhador *Orseolia oryzae* Wood Mason (Diptera: Cecidomyiidae) encontrado em áreas orizícolas da Índia, China, Nepal, Sri Lanka e Laos (Katiyar *et al.*, 2000). O percevejo-preto-do-arroz, *Scotinophara* sp. (Hemiptera: Pentatomidae), da Índia, é parasitado por várias espécies de platigastriídeos como *Telenomus triptus* Nixon, *Telenomus cyrus* Nixon, *Telenomus chloropus* (Thomson), *Telenomus gifuensis* Ashmead (Polaszek & Rajmohana, 2007) e *Telenomus oryzae* Rajmohana & Nisha. Em levantamentos de diversidade na cultura do arroz, é grande o número de representantes desta família, como por exemplo, no trabalho de Gnanakumar *et al.* (2012), na Índia, onde foram encontradas 40 espécies de parasitoides, sendo 29 pertencentes a Platygastriidae. No continente africano, tem sido registrado o parasitismo por *Platygaster diplosisae* Risbec sobre o galhador *Orseolia oryzivora* Harris & Gagné (Diptera: Cecidomyiidae), praga das áreas de arroz irrigado e de terras baixas na África Subsaariana, na Nigéria (Ogah *et al.*, 2012).

Platygastriidae parasitoides de ovos são importantes inimigos naturais de Pentatomidae, sendo que algumas espécies dessa família, especialmente os cosmopolitas *Trissolcus basalus* (Wollaston) e *T. podisi*, estão sendo usadas em muitos países no controle biológico (Clarke & Walter, 1995; Corrêa-Ferreira & Moscardi, 1996; Corrêa-Ferreira, 2002; Ehler, 2002). Estes parasitoides têm atributos biológicos e comportamentais que levam a um parasitismo densidade-dependente e regulação local das populações de pentatomídeos (Laumann *et al.*, 2008). No

Brasil, Corrêa-Ferreira & Moscardi (1996) mostraram que a liberação inoculativa de *T. basalis* pode controlar a população de percevejos.

Trissolcus spp. são parasitoides solitários que se desenvolvem dentro dos ovos dos hospedeiros. O ciclo de vida de *T. basalis* requer, em média, 17 horas para o desenvolvimento dos ovos, quatro dias para as larvas e seis dias para as pupas (Corrêa-Ferreira, 1993).

Um trabalho de Cingolani *et al.* (2012), em que é analisado em laboratório o multiparasitismo de ovos de *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) por *T. podisi* e *T. urichi*, indica esta última espécie como um melhor competidor intrínseco e que, apesar de *T. podisi* ser dominante em campo, *T. urichi* poderia estar usando outros ovos de pentatomídeos como recurso, mostrando seu potencial como controlador de vários Pentatomidae. Corrêa-Ferreira (2003) observou no Paraná, em duas safras consecutivas de soja orgânica, que a incidência de parasitismo em áreas sem liberação de parasitoides foi de 41,3% na primeira e 40,7% na segunda. Em áreas onde foram liberados os parasitoides *T. basalis* e *T. podisi*, os índices atingiram 44,3% no primeiro ano e 54,4% no segundo. Corrêa-Ferreira (2003) relatou que, geralmente, *T. basalis* e *T. podisi* são os parasitoides que apresentam maior eficiência de parasitismo. A primeira, preferencialmente, em ovos de *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) e a segunda em ovos de *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae).

Estes gêneros de parasitoides também são registrados em outras culturas, tais como no trabalho de Favetti *et al.* (2013), no qual, em um levantamento de parasitismo de ovos de *Edessa meditabunda* (Fabricius) (Hemiptera: Pentatomidae), em alface, foram encontrados 578 ovos, sendo 38,2% parasitados por *T. urichi*. Foi o primeiro registro de ocorrência em alface. Em um trabalho realizado por Santos (2008) em cultura de soja orgânica no Rio Grande do Sul, também foram encontrados parasitoides do gênero *Telenomus*, preferencialmente, em ovos de *Dichelops*

furcatus (Fabricius) (Hemiptera: Pentatomidae), enquanto que em ovos de *Chinavia* (= *Acrosternum*) *impicticorne* (Stal) (Hemiptera: Pentatomidae) e de *N. viridula* os parasitoides encontrados foram do gênero *Trissolcus*.

2.8 Problemática taxonômica e filogenética

Platygastridae pertence a superfamília Platygastroidea com 6011 espécies descritas (Hymenoptera online, 2016). As relações filogenéticas dos elementos deste grupo não são claras, sendo que, através de dados moleculares, suportam uma relação com Chalcidoidea como grupo irmão, e com dados morfológicos, indicam uma relação com Ceraphronoidea (Austin *et al.*, 2005). Apesar da relação com o grupo irmão da superfamília Platygastroidea ser ainda discutida, a sua monofilia é indiscutível (Austin *et al.*, 2005). Masner (1976), Johnson (1992) e Vlug (1995) compilaram literaturas registradas desde 1758 com espécies de Platygastroidea ocorrentes em várias partes do mundo.

Em Platygastridae, existe muita discordância a respeito da classificação na subfamília Telenominae (Kozlov, 1970; Masner, 1972; 1976; Masner & Huggert, 1979; Kozlov & Kononova, 1983). A maior parte das espécies é descrita em um dos dois gêneros, seja *Trissolcus* Ashmead (179 espécies válidas) ou *Telenomus* Haliday (652 espécies válidas), sendo, ambos, cosmopolitas. Dos gêneros restantes, 14 têm apenas uma ou duas espécies descritas (Taekul *et al.*, 2014). Alguns trabalhos apontam que não foi encontrada monofilia entre certos gêneros, entre eles *Telenomus* (Murphy *et al.*, 2007; Taekful *et al.*, 2014), no qual não foi encontrada monofilia inclusive no grupo *podisi* (Taekful *et al.*, 2014).

Estudos referentes à localização e preferência de hospedeiros têm começado a questionar se os indivíduos identificados como *T. podisi* pertencem efetivamente a uma única espécie. Em um estudo com *T. podisi* do Brasil e Estados Unidos, por exemplo, Borges *et al.* (2003)

encontraram diferenças nas respostas em relação às pistas deixadas pelos hospedeiros, tolerância ao frio, composição de hidrocarbonetos cuticulares e acasalamento entre as duas populações.

A elucidação de tais questionamentos poderá ser realizada com estudos a respeito da variação morfológica e biológica entre grupos de indivíduos e, segundo (Godfray, 1994), as técnicas moleculares ajudarão a construir relações filogenéticas entre eles. Buscando esclarecer estas questões, a sistemática filogenética pode servir a dois objetivos principais. Um deles é fornecer subsídios para uma compreensão geral da diversidade biológica, da evolução dos táxons e da modificação de caracteres. O outro é desenvolver a habilidade de propor hipóteses sobre a evolução de caracteres ou sobre as relações de parentesco entre membros de um grupo (Amorim, 2011). Segundo o mesmo autor, esta área de conhecimento deveria ser dominada por qualquer pesquisador que lide direta ou indiretamente com problemas ligados à comparação entre estruturas biológicas – de moléculas ao comportamento – de grupos diferentes de organismos.

A identificação taxonômica de fauna e flora é essencial para qualquer estudo envolvendo biodiversidade (Wägele *et al.*, 2011) e, atualmente, a falta de taxonomistas é aguda nas regiões com maiores produções de arroz, incluindo os Platygastriidae (Islam *et al.*, 2012). Antigamente, alguns países desenvolvidos e organizações internacionais proviam serviços taxonômicos à uma taxa mínima, mas a crise de financiamento cortou a atividade da maioria destes serviços, sendo este o maior empecilho para pesquisas de diversidade no arroz (Islam *et al.*, 2012). Para os autores, há uma necessidade urgente de desenvolvimento de mão de obra e instalações para o serviço de taxonomia para países em desenvolvimento que cultivam arroz.

Extrapolando as dificuldades dentro do grupo Platygastriidae para insetos em geral, entomologistas estão sendo cada vez mais solicitados em áreas de quarentena, biossegurança, agricultura e na saúde humana e animal para reconhecimento de espécies pragas, com potencial

de controle biológico e que causam doenças em humanos e animais para deliberar a respeito das filogenias e de como estas se relacionam com aspectos práticos (Gullan & Cranston, 2010). Conseqüentemente, para os autores, há um aumento na demanda por estudos de sistemática, através de caracteres morfológicos, comportamentais e técnicas moleculares para o efetivo reconhecimento das espécies.

2.9 Identificação de espécies com o uso de marcadores moleculares

A existência de um número de entidades ou táxons, que, de alguma maneira podem ser distinguidos uns dos outros, caracteriza a diversidade biológica (Amorim, 2011). Essa diversidade pode ser identificada de diferentes maneiras, podendo ser através da análise comportamental, morfológica e através da diversidade genética (Amorim, 2011).

Na diversidade genética, caracteres não aparentes podem interferir no organismo, sendo que estes cobrem os genes encontrados dentro de uma dada população de uma única espécie e o padrão de variação através de diferentes populações daquelas espécies, sendo necessária para as populações serem capazes de lidar com mudanças ambientais (Gurr *et al.*, 2012). A perda da diversidade genética é, muitas vezes, associada com a redução do valor adaptativo reprodutivo, sendo então, a matéria-prima para as mudanças adaptativas evolucionárias (Futuyma, 2002).

O estudo da diversidade genética tem sido importante em diversas linhas de pesquisas científicas na área agrônômica como: resistência de organismos a inseticidas (Xie *et al.*, 2012), estudos de cadeia alimentar (Gurr *et al.*, 2012), dispersão geográfica de espécies (Chen *et al.*, 2013; Justi *et al.*, 2016) e diferenciação de espécies crípticas (Mapondera *et al.*, 2012; Derocles *et al.*, 2016). A respeito deste último item, diversos autores vêm usando a técnica molecular para a diferenciação de espécies idênticas ou muito similares morfológicamente, tanto de pragas como de inimigos naturais (Garipey *et al.*, 2014; Uesugi *et al.*, 2016). Para o desenvolvimento

de estudos nestas áreas, são utilizados marcadores moleculares encontrados nos genes (Garipey *et al.*, 2007; Derocles *et al.*, 2014; Ronque *et al.*, 2015).

Dentre os genes utilizados como marcadores para identificação de espécies e genética de populações, encontramos o gene mitocondrial COI (citocromo oxidase I) e o ITS2 (espaço transcrito interno2 do DNA ribossomal). Ambos são marcadores com alta taxa evolutiva e o COI possui muitas variações no 3º códon de bases nitrogenadas.

Sequências do gene COI têm sido usadas para identificação de muitas espécies de insetos como *Torymus* spp. (Hymenoptera: Torymidae) (Yara, 2004), *Encarsia* spp. (Hymenoptera: Aphelinidae) (Monti *et al.*, 2005), *Calliphora vicina* Robineau-Desvoidy e *C. vomitoria* (L.) (Diptera: Calliphoridae) (Ames *et al.*, 2006), *Elachista* spp. (Lepidoptera: Elachistidae) (Kaila & Stahls, 2006), cochonilhas (Hemiptera: Pseudococcidae) (Saccaggi *et al.*, 2008), *Baetis vernus* Curtis e *B. macani* Kimmin (Ephemeroptera: Baetidae) (Stahls & Savolainen, 2008), quironomídeos (Diptera: Chironomidae) (Failla *et al.*, 2016), moscas varejeiras (Diptera: Calliphoridae) (Khullar *et al.*, 2016), *Eumerus* sp. (Diptera: Syrphidae) (Chroni *et al.*, 2016), entre outras.

Em rDNA, o ITS2 é uma escolha adequada para a identificação de espécies estreitamente relacionadas ou populações simpátricas e utilizado em espécies barcoding e tecnologias de matriz de DNA (Heraty, 2004). Apresenta a mesma funcionalidade do COI, com muitos estudos mostrando a utilidade de ITS2 na identificação de espécies (Young & Coleman, 2004), incluindo himenópteros parasitoides como *Lysiphlebus* spp. (Braconidae) (Rahimi *et al.*, 2012), *Horismenus* spp. (Eulophidae) (Kenyon *et al.*, 2015), Trichogrammatidae (Almeida & Stouthamer, 2015; Venkatesan, 2016).

Além disso, como citado anteriormente, através dos marcadores moleculares, pode-se estudar a genética de populações. A mesma investiga a conexão entre características

demográficas e a distribuição de variações moleculares e genéticas. Medindo a variação genética pode ser feita inferência a respeito da biologia de organismos (Sunnucks, 2000). No trabalho de Zepeda-Paulo *et al.* (2015), os autores avaliaram populações do parasitoide *Aphidius ervi* Haliday (Hymenoptera: Braconidae) introduzido no Chile como agente de controle de afídeos pragas. Os autores verificaram que as populações desses parasitoides sofreram o efeito gargalo moderado durante a introdução, mostrando ainda uma forte diferenciação genética entre as populações da região de origem e as de ambientes novos. Assim, processos que afetam indivíduos podem acumular efeitos nas populações podendo influenciar a especiação e, conseqüentemente, categorias taxonômicas (Sunnucks, 2000). Por isso, a maioria dos taxonomistas, além das características morfológicas, incorporam análises filogenéticas em seus trabalhos, buscando uma taxonomia integrativa a fim de elucidar questões referentes às espécies estudadas (Schlick-Steiner *et al.*, 2010; Johnson, 2011). Dessa maneira, essa ferramenta poderá auxiliar a esclarecer as dúvidas a respeito da filogenia assim como a delimitação de espécies do grupo em questão.

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3 ARTIGO 1

**Riparian forest fragments in rice fields under different management: differences by
hymenopteran parasitoids diversity ***

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1 **Riparian forest fragments in rice fields under different management:**
2 **differences by hymenopteran parasitoids diversity**

3
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9 *Running title: Riparian forest fragments in rice fields*

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11
12 **Abstract** 1 Hymenopteran parasitoids are important biological control agents in
13 agroecosystems, and their diversity can be increased with habitat heterogeneity.
14 2 The objective of this study was to evaluate the contribution of the presence of
15 fragments of natural vegetation (riparian forest) near rice-growing areas and the
16 influence of different management of the crop (organic and conventional) on the
17 diversity of families of hymenopteran parasitoids, through distance gradients,
18 and on the vegetative and reproductive stages of the crop.
19 3 The work took place in two irrigated rice crops, one with organic management
20 (O.M.) and another with conventional management (C.M.), in the municipality
21 of Nova Santa Rita, RS, Brazil, during the 2013/2014 and 2014/2015 seasons.
22 The parasitoids were collected with Malaise trap arranged at different distances
23 in relation to the riparian forest surrounding the rice crop in both places.
24 Specimens were collected twice a month from seeding until the rice harvest.
25 Average abundance between management, distances and rice development were
26 compared.
27 4 The most abundant families were Platygasteridae, Mymaridae, Encyrtidae,
28 Eulophidae and Trichogrammatidae, their abundances changing over the
29 seasons. Parasitoid average abundance was significantly higher on O.M. only in
30 the second season.
31 5 There was a negative correlation between distance from native vegetation and
32 parasitoid abundance in C.M. areas.
33 6 The phenological stages of rice affected the composition of the parasitoid
34 assemblage on site.

35
36 **Keywords** Conservative biological control, parasitoids, rice, habitat heterogeneity, native
37 vegetation.

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46 Introduction

47 Rice (*Oryza sativa* L.) is the second most cultivated cereal in the world, occupying an area of
 48 158 million hectares and corresponds to 29% of the grains used for human consumption
 49 (SOSBAI, 2014). The rice plant can be damaged by various phytophagous groups (EMBRAPA
 50 CLIMA TEMPERADO, 2009). To control these pests, chemical insecticides based on
 51 neonicotinoid, diamide, pyrethroid and carbamate are mainly used in conventional management
 52 (AGROFIT, 2016). On the other hand, the production of organic rice is based on eliminating
 53 the use of synthetic chemical inputs such as fertilizers, pesticides and growth regulators, and
 54 promoting alternative technologies such as mechanization, tillage and fertilization in the
 55 management of pests, including weeds, diseases and insects (EMBRAPA CLIMA
 56 TEMPERADO, 2009). In addition, the maintenance of permanent preservation areas and the
 57 mitigation of anthropogenic pressure on natural and modified ecosystems are environmental
 58 aspects that organic rice production systems seek to obtain certification (EMBRAPA CLIMA
 59 TEMPERADO, 2009).

60 Parasitoids are one of the most important groups of biological control agents in the
 61 control of pest populations in agricultural systems, both by the natural occurrence of the species
 62 in the environment and by their use in classic biological control programs (Hanson & Gauld,
 63 2006). Parasitoid diversity associated with different cultivation systems is determined by
 64 environmental, biological, and management factors (Chay-Hernandez *et al.*, 2006). In large
 65 monocultures, the diversity can be suppressed by pesticides, simplification of vegetation, and
 66 other environmental disturbances (Altieri, 2003). In less disturbed agricultural ecosystems, the
 67 diversity of these agents appears to be related to the diversity of crops, weeds, ground cover and
 68 native vegetation next to cultivated systems (Liere *et al.*, 2015).

69 In this context, this study evaluated the contribution of the presence of fragments of
 70 Riparian forest near irrigated rice areas and the influence of different management of the crop
 71 (organic and conventional) diversity of families of Hymenopteran parasitoids, through distance
 72 gradients in rice production systems, and on the vegetative and reproductive stages of plants.
 73

74 Material and Methods

75 Study area

76 The study was carried out in the municipality of Nova Santa Rita, RS, in the metropolitan region
 77 of Porto Alegre in the phytogeographic region of the Jacuí watershed, during the 2013/2014 and
 78 2014/2015 seasons. In this region many native trees species occur such as ‘maricá’ (*Mimosa*
 79 *bimucronata* (DC) Kuntze), ‘corticeira’ (*Erythrina falcata* Benth.), fig tree (*Ficus luschnatiana*
 80 (Miq.) Miq.), ‘angico’ (*Parapiptadenia rigida* (Benth.) Brenan), Surinam cherry (*Eugenia*
 81 *uniflora* L.), ‘branquilha’ (*Sebastiania commersoniana* (Baill.) L.B. Sm. & Downs), ‘açoi-
 82 cavalo’ (*Luehea divaricata* Mart. & Zucc.) and many others (COPTec, 2010). The vegetation
 83 near to irrigated rice area is a riparian forest.

84 *Organic rice crop.* The irrigated rice area with organic management (O.M.) is part of the Capela
 85 Settlement (Landless Rural Workers Movement Settlement) in Nova Santa Rita. It has a total
 86 area of 2169.37 hectares (INCRA, 2007), of which 580 ha belong to COOPAN (Agricultural
 87 Production Cooperative Nova Santa Rita). Of these, 220 continuous hectares are allocated for
 88 organic rice, which is certified by the Ecological Market Institute (Instituto de Mercado
 89 Ecológico - IMO) (Lanner, 2011). In the settlement an area of 273.52 hectares (13.68% of the
 90 total) is designated as a permanent preserve (native forest). In the organic rice field, the levees
 91 are maintained with wild vegetation.

92 In the first season (December, 2013 to April, 2014), five points were defined for the
 93 installation of traps centered around this coordinate 29° 47' 16.62" S and 51° 21' 00.91" W. In
 94 the area where the traps were installed, it was used the pre-germinated planting system with
 95 cultivar EPAGRI 108 which has late cycle, lodging resistance and indirect iron toxicity. It is
 96 moderately resistant to panicle blast and stands out for its excellent grain quality and high
 97 reproductive potential (SOSBAI, 2014).

98 In view of the difficult access to the area in the first year, another five points were defined
 99 in the second year (November, 2014 to March, 2015) for the installation of traps centered around
 100 this coordinate 29° 46' 48.90" S, 51° 21' 48.38" W. The management of the area was the same
 101 as in the first year.

102 In the first year, the traps were separated 100 m from each other, in a distance gradient
 103 starting from inside the forest to the middle of the crop, in a straight line (Figure 1). In the second
 104 year, the distance was extended to 200 m. Each trap site was named by the location of the
 105 Malaise trap, being O5 (Spot 5 - inside the forest), O4 (Spot 4), O3 (Spot 3), O2 (Spot 2), O1
 106 (Spot 1 - greatest distance from forest) (Figure 1).

107 *Conventional rice crop.* The irrigated rice area with conventional management (C.M.) is located
 108 in the 'Gaúcha' Farm, a private property of 300 hectares approximately 2.5 km distant from the
 109 organic management area and bordered to the southwest by the Caí River. Tillage is the planting
 110 system, and the cultivar used was IRGA428, which has a medium cycle, high productivity
 111 potential, tolerance to herbicides, tolerance to toxicity from excess iron, is moderately
 112 susceptible to leaf blast and susceptible to blast on panicle (SOSBAI, 2014).

113 The conventional management adopted by the producer follows the IRGA
 114 recommendations, with the use of chemical inputs and application of agricultural pesticides
 115 according to the annual manuals for rice management (SOSBAI, 2012). For insect collecting,
 116 the experimental design was the same of the organic management area (Figure 1), with the
 117 points set between 29° 46' 16.65" S and 51° 21' 43.31" W, the same in both seasons. In the
 118 conventional rice field, the levees are maintained without wild vegetation by chemicals
 119 spraying.

120 As in the O.M. area, in the first year (December, 2013 to April, 2014), the traps were
 121 from each other by 100 m, in a straight line, and in the second (November, 2014 to March,
 122 2015), the distance was extended to 200 m. Each spot also was named by the location of Malaise
 123 trap, being C5 (Spot 5), C4 (Spot 4), C3 (Spot 3), C2 (Spot 2), C1 (Spot 1).

124 In the first season regulators insecticides were used (buprofezin and benzylurea) and in
 125 the second season were used non-selective (neurotoxic) insecticides (neonicotinoids and
 126 pyrethroids) (farmer personal communication Mr. Denis).

127 *Hymenoptera sampling and identification*

129 For capturing the parasitoids, five Malaise traps (Townes, 1972) were placed in the levees of
 130 each area (Figure 1). The collection method was improved, by putting PVC pipes cut in half to
 131 simulate a "gutter" in each trap, so that insects which have not reached the collecting head could
 132 drop in the "gutter" (containing water and detergent) located on the ground, comprising the
 133 entire length of the trap (Gullan & Cranston, 2008).

134 Samples were collected every two weeks during two rice seasons, 2013/2014 and
 135 2014/2015, from seeding to harvest. The traps remained mounted in the points for 24 hours and
 136 thereafter the vials with the collected insects were taken. These were transferred to 70% alcohol,
 137 labeled with the collection site, and transported to the Laboratory for Biological Control of
 138 Insects (CBLAB) of UFRGS. In the laboratory, the samples were screened using a

139 stereomicroscope Nikon SMZ445, selecting the hymenopteran parasitoids. The identification of
 140 families followed the classification adopted by Goulet & Huber (1993) and Sharkey (2007). The
 141 specimens were deposited in the Education Collection at Laboratory for Biological Control of
 142 Insects (CBLAB) of UFRGS in Agronomy University.

143

144 *Statistical analyses*

145 Considering the extent of the two areas and their characteristics, it was impossible to make
 146 replicas of the samples, so that evaluations were considered exploratory.

147 The average capture of parasitoids was compared between areas and between crop
 148 development periods, considering the four traps installed inside the crop as pseudoreplicas,
 149 through Shapiro-Wilk normality test and analysis of variance (Kruskal-Wallis test).

150 The rice developmental stages were identified according to Counce *et al.* (2000). Here
 151 we named indicated O.V or C.V (organic or conventional vegetative stage of rice) and O.R or
 152 C.R (organic or conventional reproductive stage of rice), respectively.

153 For evaluating the abundance of parasitoids over the gradient of distance from traps in
 154 relation to the native vegetation, Pearson correlation test was used through the BioEstat 5.3
 155 program (Ayres *et al.*, 2011).

156 Correspondence Analysis (CA) and Bray-Curtis Cluster Analysis plots were built
 157 through the PAST program (Hammer *et al.*, 2001). Plots are constructed with the main
 158 components of lines and columns allowing visualization of the relationship between groups,
 159 where the proximity of the points relating to the line (parasitoid families) and column (spots and
 160 rice stages) indicates association and the distance indicates repulsion (Greenacre & Hastie,
 161 1987). The Cluster Analysis groups the data in a dendrogram, in which the level of similarity (or
 162 dissimilarity) is indicated on the vertical scale. On the horizontal axis, the sample elements are
 163 reported in a convenient order for the clustering (Mingoti, 2005).

164

165 **Results**

166 *Faunistic data*

167 *First Season (2013-2014)*. In the first season, 1,104 individuals were collected in the rice area
 168 with organic management (O.M.), distributed in 21 families; and 860 individuals in the rice area
 169 with conventional management (C.M.), distributed in 18 families.

170 The total average of captured individuals/trap/day was 30.3 ± 5.42 in the O.M. area and
 171 22.3 ± 7.32 in the C.M. area in the first season, with no significant difference ($H = 2.08$, $df = 1$,
 172 $p = 0.1$).

173 Regarding the development stage of rice within each area, in the O.M., the total capture
 174 average was significantly lower in the vegetative stage (24.45 ± 6.96) than in the reproductive
 175 (40.1 ± 4.74) ($H = 5.33$; $df = 1$; $p = 0.02$). In the C.M., however, there was no significant
 176 difference between the vegetative (16.8 ± 10.58) and reproductive stages (29.31 ± 4.06) ($H = 3$;
 177 $df = 1$; $p = 0.08$).

178 Considering the same development stage between areas, in the reproductive stage, a
 179 significantly higher average of captures (40.08 ± 4.74) in the O.M. was observed than in the
 180 C.M. over this period (29.31 ± 4.06) ($H = 5.33$; $df = 1$; $p = 0.02$). In the vegetative period, there
 181 was no significant difference between the O.M. (24.45 ± 6.95) and the C.M. (16.8 ± 10.58) (H
 182 $= 1.33$; $df = 1$; $p = 0.24$).

183 The most abundant families in the O.M. were Platygasteridae and Mymaridae. In the
 184 C.M., the most abundant were Encyrtidae and Platygasteridae (Table 1).

185 *Second Season (2014-2015)*. In the second year's season, 1,064 parasitoids were collected in
 186 the O.M. area, divided into 19 families, and 389 individuals were collected in the C.M. area,
 187 distributed in 16 families.

188 The total average of trapped individuals in the O.M. area was 25.38 ± 6.85 trap/day,
 189 significantly higher than in the C.M. area (8.41 ± 3.40) ($H = 5.33$; $df = 1$; $p = 0.02$).

190 In a comparison of developmental stages within each area, the vegetative stage ($17.33 \pm$
 191 3.12) averaged significantly lower captures than the reproductive (29.41 ± 9.36) in the O.M. (H
 192 $= 4.08$; $df = 1$; $p = 0.04$), however, there was no difference between stages in the C.M.
 193 (vegetative: 10.06 ± 2.46 and reproductive: 6.7 ± 4.59) ($H = 2.08$; $df = 1$; $p = 0.14$).

194 Comparison of trap catches in the same development stage of rice between areas showed
 195 that in the vegetative stage the O.M. averaged significantly higher captures (17.33 ± 3.12) than
 196 the C.M. (10.06 ± 2.46) ($H = 5.33$; $df = 1$; $p = 0.02$), as well as in the reproductive stage, with
 197 significantly higher captures in the O.M. (29.41 ± 22.54) than in the C.M. (6.7 ± 2.28) ($H =$
 198 7.04 ; $df = 1$; $p = 0.008$).

199 The most abundant families in the O.M. were Eulophidae, Mymaridae and
 200 Platygasteridae. In the C.M., they were Platygasteridae, Mymaridae and Trichogrammatidae
 201 (Table 1).

202

203 *Riparian forest effects on parasitoid abundance in rice fields*

204 In the first season, there was a negative correlation between the number of insects captured and
 205 the distance from the forest area for the C.M. (Figure 2A), and 92% of this variation may be
 206 explained by the distance in relation to the forest next to the planting area. In the O.M., however,
 207 this correlation could not be detected.

208 In the second season, there was no correlation between the distance gradients in any of
 209 the areas (Figure 2B), even with increased distance between traps.

210

211 *Association between sampling spots, management, phenological stages and parasitoid families*

212 Through correspondence analysis, the points were represented on two axes, indicating that the
 213 association between lines (parasitoids families) and columns (spots and rice stages) is two-
 214 dimensional, 60% of the relationship being explained in these two axes in season 1 and 69% of
 215 the relationship being explained in season 2.

216 In the first season, samples are mixed on both sides of the x and y axes, showing similar
 217 composition between areas (Figure 3). Nonetheless, some families had higher relationships with
 218 certain sampling points and phenological periods of the plant. Mymaridae and
 219 Trichogrammatidae, showing confluence with the sampling areas of O.M. and both the
 220 phenological stages and with the sampling areas of C.M. and its vegetative phenological stage.
 221 On the other hand, Encyrtidae showed greater relationship with sampling areas of the C.M. in
 222 the reproductive stage. The points Organic 1 (O1) and Organic 2 (O2) were the most dissimilar
 223 from the others.

224 In the second season (Figure 4), the samples of the organic and conventional
 225 managements showed dissimilarities between them, since they appear to be distributed on
 226 distinct sides, with different families relating to each area. Nevertheless, the most abundant
 227 families exhibit similarities to each other along the y axis. Platygasteridae, Mymaridae and
 228 Encyrtidae appear to be in the same position on the y axis, showing to have the same importance
 229 for the composition to which they belong, being Platygasteridae, on the right side, related to

230 C.M.; Eulophidae, on the left side, related to O.M.; and Mymaridae, in center, related to both
231 areas.

232 Differences between the composition and abundance of families are perceived between
233 the two sampling years for the areas through cluster analysis. The high cophenetic correlation
234 in the two years (0.94 and 0.85, respectively) confirms the adjustment of data to the Bray-Curtis
235 model (Figures 5 and 6).

236 In the first season, there was similarity between the different sampled points, regardless
237 of the adopted management. Considering a cut-off point in 60%, only the organic management
238 points (O1 and O2) were dissimilar from the others (Figure 5).

239 In season 2, two clusters are distinct, clearly pointing out similarities between the
240 managements used (Figure 6). All points within the crop of the organic management area
241 showed about 75% similarity. The points relating to the conventional management area showed
242 similarity ranging from 60% to 70%. The lowest percentage of similarity (55%) was that of the
243 organic 5 (O5), presenting itself as independent of the others.

244

245 Discussion

246 A comparison of the results between the two seasons (2013/2014 and 2014/2015) showed that
247 in the O.M. area the abundance of individuals was similar despite the distance between sampling
248 points. However, in the C.M., there was much less abundance in the second season. This may
249 be due to the use of non-selective (neurotoxic) insecticides (neonicotinoids and pyrethroids)
250 applied to the crop in 2014/2015, different from the first season, when growth regulators
251 insecticides were used (buprofezin and benzylurea) (farmer personal communication Mr.
252 Denis). This difference occurs because the products that act on chitin synthesis are either
253 ecdysteroid receptor agonists or are generally more selective to natural enemies and other
254 beneficial insects such as bees, because they act only on immature stages, having a smaller
255 impact on adults (Bastos *et al.*, 2006).

256 Differences in the abundance of parasitoids between crop development stages in the
257 O.M. suggests that in this system the phenological stage of the crop influences the presence of
258 natural enemies. These are possibly related to the hosts, since different pests are related to the
259 distinct development stages of rice. Hence, the higher abundance of Mymaridae, Platygasteridae
260 and Eulophidae was expected, given that these families are associated with rice pests in many
261 countries, as Delphacidae in Asia (Gurr *et al.*, 2011), Pentatomidae (Maciel *et al.*, 2007; Idalgo
262 *et al.*, 2013) and Lepidoptera in Brazil (EMBRAPA ARROZ E FEIJÃO, 2008).

263 In Brazil, Platygasteridae, mainly the genera *Telenomus* and *Trissolcus*, are important
264 biological control agents of Pentatomidae in rice crops (Maciel *et al.*, 2007; Riffel *et al.*, 2010).
265 Their high abundance within the rice fields is associated with resource availability in the crop,
266 therefore, available hosts. Pentatomidae are common both in the vegetative and reproductive
267 stages of rice (SOSBAI, 2014), accounting for the great number of Platygasteridae in both
268 development stages of the crop.

269 Other highlighted families were Eulophidae (O.M.) and Encyrtidae (C.M.). For rice,
270 Gurr *et al.* (2011) listed *Aprostocetus formosanus* (Timberlake, 1921) (Eulophidae) as
271 parasitoids of Delphacidae in the Philippines, Vietnam, Malaysia and Thailand. Other authors
272 have also reported individuals of Eulophidae in rice crops (Nacro *et al.*, 1997; Williams *et al.*,
273 1999; Bayegan *et al.*, 2015), and Gumovsky *et al.* (2006) described a new species of Eulophidae,
274 genus *Closterocerus*, parasitoid of *Dicladispa armigera* (Olivier, 1808) (Coleoptera:
275 Chrysomelidae), a major rice pest in Southeast Asia and Australasia. In rice cultivation in Rio

276 Grande do Sul, Eulophidae also was the most abundant parasitoid family and the phytophagous
277 registered were Curculionidae, Pentatomidae, Delphacidae and Cicadellidae (Fritz *et al.*, 2011).

278 The second most abundant family in C.M., Encyrtidae has records of hosts of rice pests
279 in various regions of the world. For example, Kraker *et al.* (1999) noted the parasitism of
280 *Cnaphalocrocis medinalis* (Guenée, 1854) and *Marasmia* spp. (Lepidoptera: Pyralidae), rice
281 pests increasing in abundance since 1960 in many Asian countries, by *Copidosomopsis*
282 *nacoleiae* (Eady, 1960) (Encyrtidae) (Khan *et al.*, 1988; Dale, 1994). *Ooencyrtus nezarae* Ishii,
283 1928 and *Xenoencyrtus niger* Riek, 1962, both species introduced in the Neotropics, are
284 parasitoids of Pentatomidae eggs (Hanson & Gauld, 2006). In Brazil, *Ooencyrtus submetallicus*
285 (Howard, 1886) was recorded as a parasitoid of *T. limbativentris* eggs in rice crops in Maranhão
286 (Maciel *et al.*, 2007).

287 Mymaridae, a family among the most abundant in both seasons, especially in the
288 vegetative stage in the first season, and reproductive stage in the second one, is known to attack,
289 mostly, Auchenorrhyncha (Hemiptera). Nonetheless, this parasitoid family also attacks
290 Coleoptera, Orthoptera and other Hemiptera (Hanson & Gauld, 2006). These small parasitoids
291 of eggs are recorded attacking leafhoppers, which are rice pests, of Delphacidae present in Asia
292 and Oceania (India, China, Japan, Malaysia, Singapore, Vietnam, Sri Lanka, the Philippines,
293 Thailand, Korea, Taiwan, Indonesia) (Gurr *et al.*, 2011). In Brazil, species like *Tagosodes*
294 *orizicolus* (Muir, 1926) (Hemiptera: Delphacidae), *Graphocephala* sp. and *Estianus* sp.
295 (Hemiptera: Cicadellidae) are recorded in rice (Didonet *et al.*, 2001). The presence of
296 Mymaridae species can be related with leafhopper on the crop.

297 In the C.M., the phenological stage did not influence the parasitoid composition. Other
298 factors such as the habitat heterogeneity or the crop management may have induced the
299 characteristics of the assembly, which has also been recorded in other studies (Tanaka *et al.*,
300 2000; Gurr *et al.*, 2011; Zaho *et al.*, 2015).

301 The clear correlation between the distance from the native forest to the crop in relation
302 to the abundance of parasitoids observed in the C.M. in the first season suggests the importance
303 of this area in the presence of these natural enemies in the crop. The role of these forest
304 fragments in maintaining the richness and abundance of parasitoids has been described by
305 Corbett and Plant (1993), Romeis *et al.* (2005) and Da Silva *et al.* (2016). In the second season,
306 this correlation was not observed due to the large decrease in the abundance of parasitoids,
307 already discussed with relation to the management.

308 In the organic management area, however, the correlation between the gradient of
309 distance was not demonstrated in any of the seasons. This can possibly be explained by the fact
310 that it is an organic management area, not receiving chemical inputs, which negatively affects
311 the presence of parasitoids, even in central areas of the monoculture. Moreover, as in the O.M.
312 there is wild levees vegetation, including the presence of flowering species that increase the
313 richness of parasitoids (Simões-Pires *et al.*, 2016). Therefore, the abundance may not be so
314 dependent on the presence or proximity of the preserved area. Levees can serve as corridors
315 extending the distances traveled by parasitoids (Gurr *et al.*, 2011).

316 In Asia, it is found that even with the harmful effects of insecticides, the level of
317 parasitism in Delphacidae increases when the habitat is manipulated ecologically, emphasizing
318 that adjacent areas can support and allow the persistence of parasitoids even if the rice crops are
319 not present (Gurr *et al.*, 2011). The levees also allow a greater richness of phytophages, both of
320 pest species attacking the culture in the vegetative period and of those with no agricultural
321 importance and that can serve as alternative hosts for the parasitoids also found there. This was
322 demonstrated in a study with a *Zizania caduciflora* (Turcz.) (Canadian rice) planting,
323 surrounding a rice crop in China (Yu, 2001). This study shows that this surrounding vegetation

324 supports *Saccharosydne procerus* Matsumura, 1924, a Delphacidae that does not develop in rice
 325 (Yu, 2001). However, this leafhopper is parasitized by *Anagrus optabilis* (Perkins, 1905) which
 326 is also an important parasitoid of *Nilaparvata lugens* (Stål, 1854) a rice pest (Zheng *et al.*, 1999).

327 The levees have been neglected as a major reserve of plants and resources for natural
 328 enemies. Nectar can maximize the longevity and fecundity of parasitoids, and pollen may allow
 329 other natural enemies during the period of prey shortage (Zheng *et al.*, 2003b). Pest insects and
 330 natural enemies were more abundant and rich in the Philippines in rice fields surrounded by
 331 vegetated levees than in rice fields without this feature (Marcos *et al.*, 2001; Gurr *et al.*, 2011).
 332 Furthermore, females of *Copidosoma koehleri* Blanchard, 1940 (Encyrtidae) with access to
 333 honey were more enduring and parasitized significantly more eggs of *Phthorimaea operculella*
 334 (Zeller, 1873) (Lepidoptera: Gelechiidae) than the wasps with access only to water (Baggen &
 335 Gurr, 1998). This may be an important advantage, as parasitoids in large monocultures need to
 336 forage over large distances, leading to high costs in terms of time and energy (Powell, 1986),
 337 affecting their distribution within the crop and consequently the distribution and population of
 338 pests (Baggen & Burr, 1998).

339 We emphasize that this study aimed to evaluate the differences in the complexity of
 340 habitats not only with regard to the abundance, but also taking into account other aspects such
 341 as richness and distribution of species and individuals. This was also reported by Edwards &
 342 Wratten (1981), for whom more complex communities provided a broader spectrum of
 343 ecological niches and sustained larger and more diverse populations of predators and parasitoids
 344 than the simpler ones. Thus, through correspondence analysis, one can see the relationship
 345 between sampling points, rice development stages and management systems, with regard to the
 346 diversity and abundance of families of parasitoid Hymenoptera. In addition, cluster analysis
 347 combines the samples according to the similarities between them.

348 In the first season, generally samples are mixed on both sides of the axes, showing a
 349 relationship between them. This may be because, although with different managements, both
 350 areas are inserted in the same ecosystem with similar adjacent vegetation (COPTec, 2010) and
 351 these environments with native vegetation can increase arthropod diversity, in general, and
 352 parasitoid diversity, more specifically, in the cultivated areas (Ferreira *et al.*, 2014; Da Silva *et al.*,
 353 2016). Notwithstanding, in the second season samples of the organic and conventional
 354 managements showed dissimilarities as they appear to be distributed on different sides in the
 355 correspondence analysis chart. This may be related to a non-restrictive treatment of chemical
 356 inputs in the C.M., leading to a compositional difference between the organic management and
 357 the conventional management areas. The similarity of the points in the areas, disassociating the
 358 managements, was expected, considering that the extensive use of insecticides affects not only
 359 the pests but also beneficial insects (Tanaka *et al.*, 2000; Gangurde, 2007), which leads to
 360 differences in their composition.

361 In the first sampling year, the sampling point organic 2 (O2) had a similarity lower than
 362 32% with other samples, being the point with greatest dissimilarity. This may be due to
 363 differences between the number of individuals being the point with the lowest abundance of
 364 platygastriids and trichogrammatids, besides has the greatest number of ceraphronids, which is
 365 the family with greater similarity to the O2. The sampling point O1 was also different from the
 366 others, but because of the composition of families, having more singletons (those species in
 367 which only one individual has been collected) and doubletons (only two individuals collected)
 368 than the others (6). The presence of rare species in an assembly provides higher diversity indices
 369 (Magurran, 2011) and may be responsible for the dissimilarities identified.

370 In season 2, the lowest percentage of similarity (55%) was from the sampling point O5,
 371 gathering together the cluster of samples of the conventional management. The point O5 had a

low number of mymarids and eulophids in relation to the other points and greater abundance of families Ichneumonidae, Bethyridae, Ceraphronidae and Eupelmidae. This similarity can also be seen through the correspondence analysis, which shows similarity between the point and these families. The association of a family to a certain point should be linked to the use of resources by the group, both of hosts (Plecasa *et al.*, 2014) and alternative resources like nectar, pollen or refuge (Gurr *et al.*, 2011). Thus, an assessment of the phytophagous fauna present in the area could give an indication of what contributes to the composition of species of parasitoids. This aspect, however, was not evaluated in this study.

Other points showed similar compositions, and families Platygasteridae, Mymaridae, Braconidae, Figitidae, Encyrtidae, Trichogrammatidae and Pteromalidae were the ones which contributed most to this in the first season. In the second, the families that contributed most were Platygasteridae, Mymaridae, Eulophidae, Encyrtidae, Braconidae and Figitidae.

The differences showed in this research regard to distance gradients of the native riparian forest area in relation to rice crop highlight the importance of this refuge for parasitoids diversity, mainly in the conventional management area, where there is no levees vegetation. In the organic management, it influences is not seen, probably due to levees vegetation presence, that can provide refuge, shelter and ecological corridors for natural enemies. The chemical insecticides in rice crops can change parasitoids diversity through changes of selective insecticides to natural enemies (growth regulators) to non-selective (neurotoxic). The phenological stage of rice influences the parasitoids diversity on site and it must be related to different hosts, oftentimes phytophagous insects, in each crop development.

393

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397

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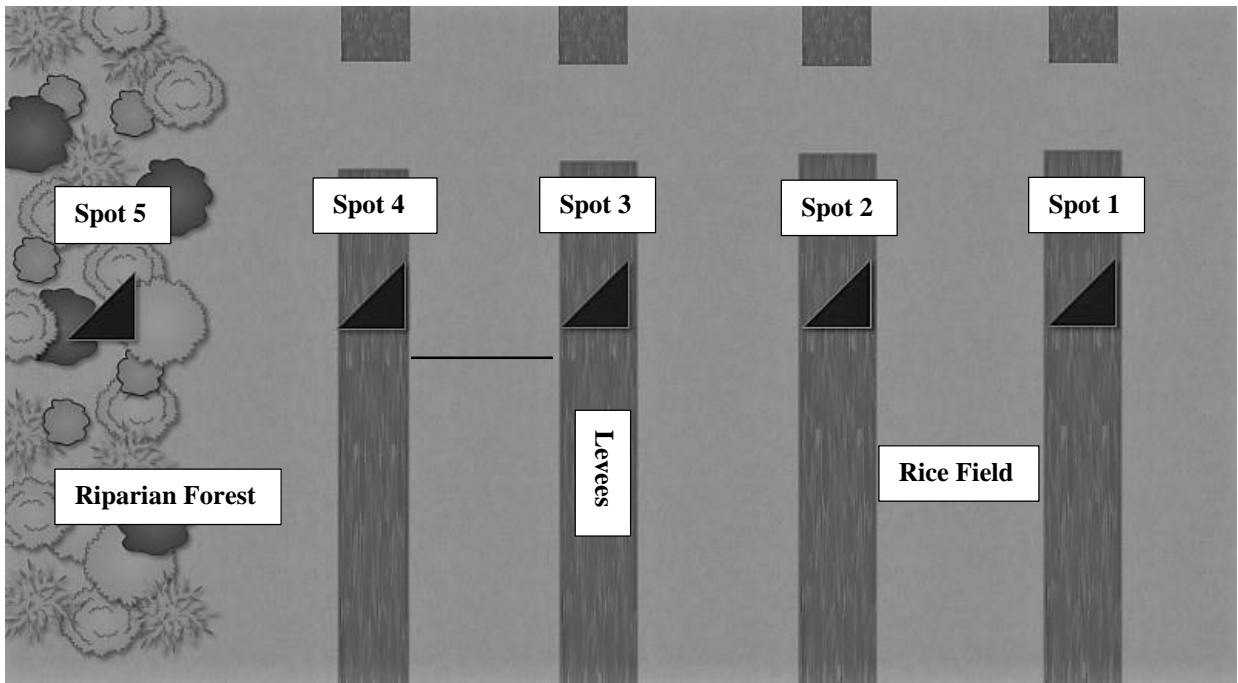
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608 Table 1. Hymenoptera parasitoids families sampled in the first (2013/2014) and second crop
 609 season (2014/2015) over vegetative stage (Veg) and reproductive stage (Rep) of rice crop in
 610 organic management area (O.M.) and conventional management area (C.M.), Nova Santa Rita,
 611 RS.

Family	1° Crop Season						2° Crop Season					
	O.M.			C.M.			O.M.			C.M.		
	Veg	Rep	Fr (%)	Veg	Rep	Fr (%)	Veg	Rep	Fr (%)	Veg	Rep	Fr (%)
Platygastridae	131	137	24.2	81	94	20.3	59	120	16.8	60	55	29.5
Mymaridae	106	79	16.7	102	43	16.8	47	186	21.9	37	43	20.5
Eulophidae	49	81	11.8	12	15	3.1	58	212	25.4	17	10	6.9
Encyrtidae	54	67	10.9	27	182	24.2	12	50	5.8	15	18	8.5
Trichogrammatidae	79	54	12	56	78	15.5	14	38	4.9	21	29	12.8
Ichneumonidae	45	27	6.5	12	5	2	25	65	8.5	6	6	3.1
Ceraphronidae	16	11	2.4	18	50	7.9	8	13	2	7	6	3.3
Braconidae	21	28	4.4	16	20	4.2	16	36	4.9	20	7	6.9
Figitidae	19	11	2.7	13	0	1.5	9	20	2.7	8	3	2.8
Diapriidae	9	5	1.3	8	1	1	3	6	0.8	3	1	1
Bethylidae	16	3	1.7	2	1	0.3	7	5	1.1	4	2	1.5
Chalcididae	8	4	1.1	5	2	0.8	1	13	1.3	3	1	1
Eupelmidae	9	1	0.9	2	2	0.5	2	10	1.1	1	1	0.5
Pteromalidae	3	0	0.3	5	2	0.8	4	9	1.2	0	1	0.3
Aphelinidae	5	5	0.9	1	1	0.2	0	3	0.3	1	0	0.3
Chrysididae	4	1	0.5	0	0	0	1	2	0.3	0	0	0
Evaniidae	3	2	0.5	0	0	0	1	2	0.3	0	0	0
Signiphoridae	0	1	0.1	0	0	0	0	4	0.4	0	0	0
Dryinidae	2	0	0.2	1	0	0.1	0	3	0.3	0	0	0
Torymidae	3	0	0.3	0	0	0	0	0	0	0	0	0
Megaspilidae	0	0	0	1	1	0.2	0	0	0	0	0	0
Eurytomidae	3	2	0.5	1	0	0.1	0	0	0	0	0	0
Proctotrupidae	0	0	0	0	0	0	0	0	0	0	3	0.8
TOTAL	585	519	100	363	497	100	267	797	100	203	186	100

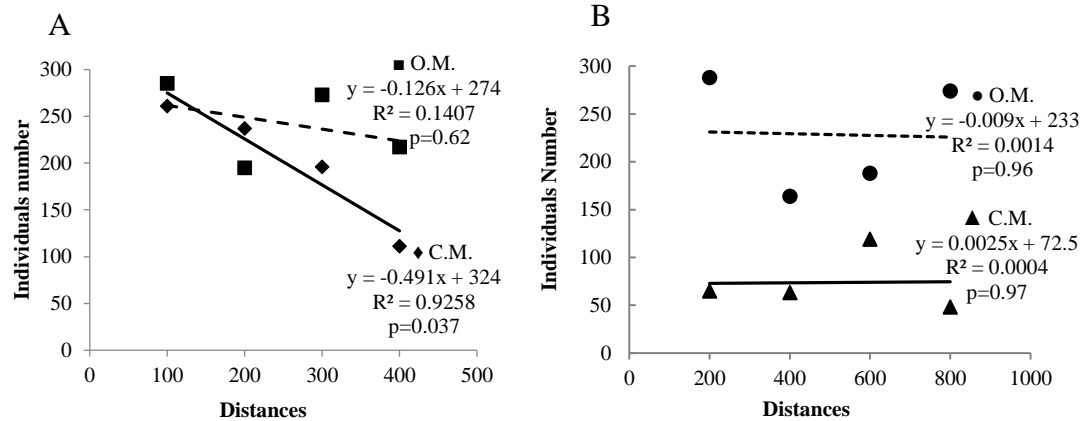
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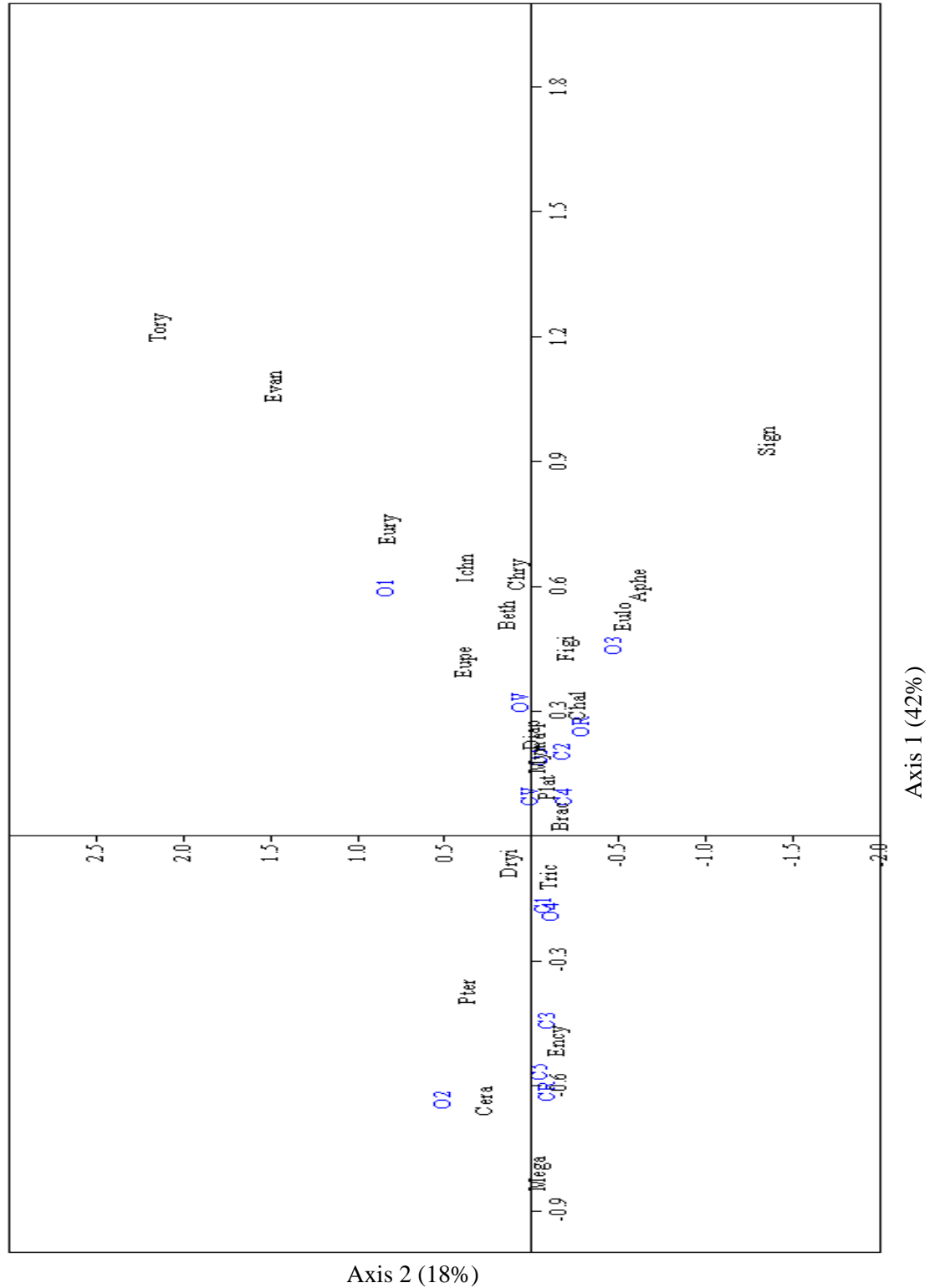
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Figure 1. Layout showing Malaise traps (▲) position on rice field and riparian forest in Organic Management Area (O.M.) and Conventional Management Area (C.M.), in Nova Santa Rita, RS. (—) Distance between traps (100m first season; 200m second season).



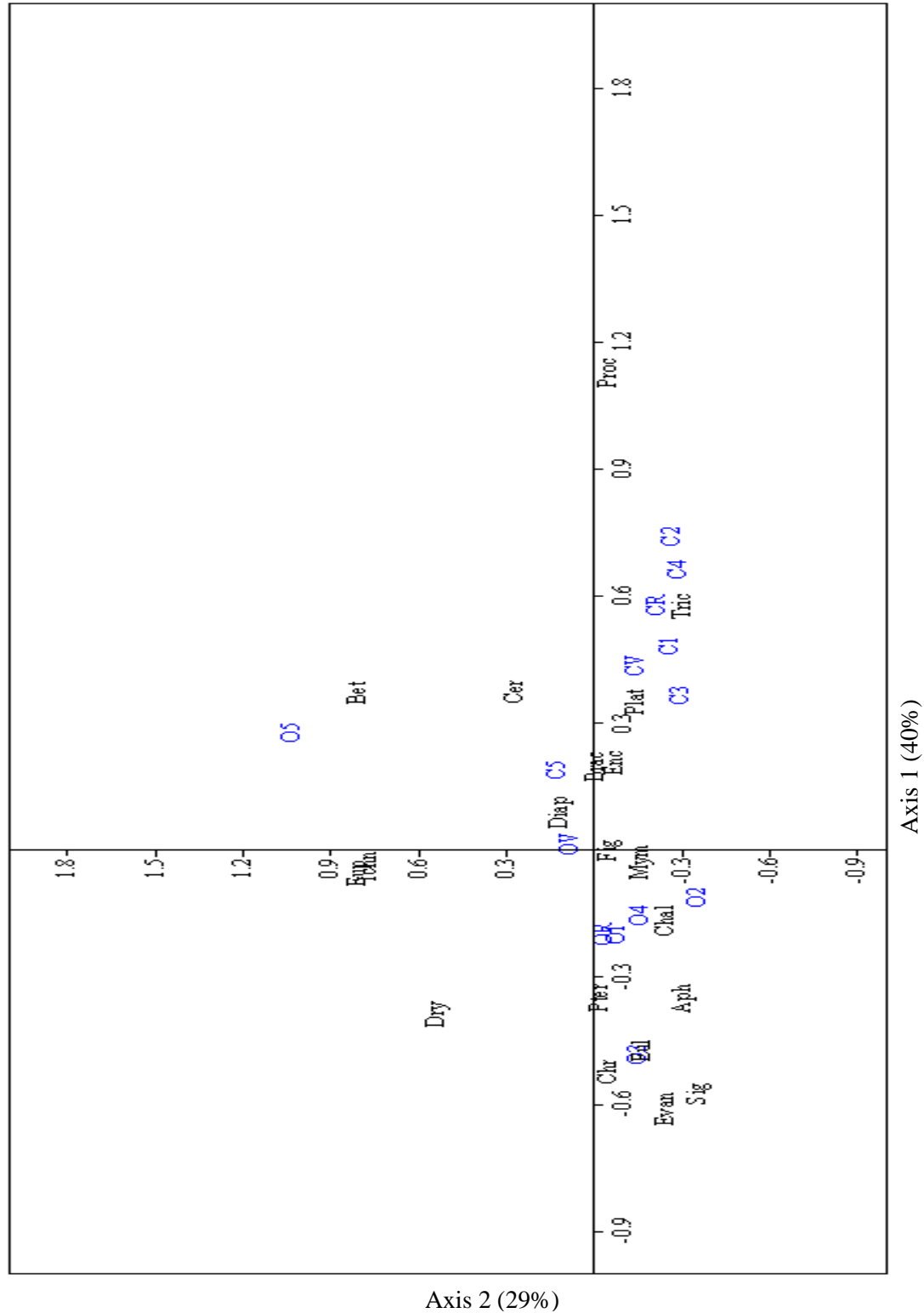
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Figure 2. Correlation between samples spots with 0, 100m, 200m, 300m e 400m distances in first crop season (A) from riparian forest (O.M. $p > 0.05$ e C.M. $p < 0.05$. Pearson Test). Correlation between samples spots with 0, 200m, 400m, 600m e 800m distances in second crop season (B) from native vegetation (O.M. $p > 0.05$ e C.M. $p > 0.05$. Pearson Test).



631

632 Figure 3. Correspondence Analyses. Relation between sample spot in each rice crop
 633 development stages (V= vegetative; R= reproductive) in each point of the areas with
 634 organic management (O) and conventional (C) and parasitoids families in the first
 635 season (2013/2014).



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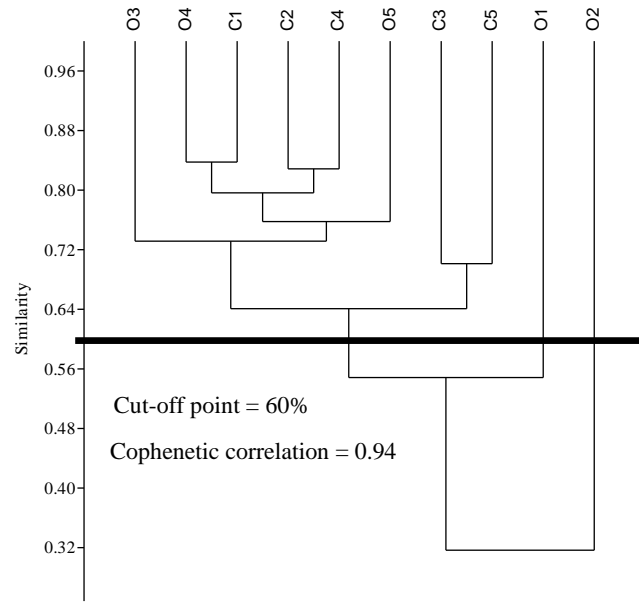
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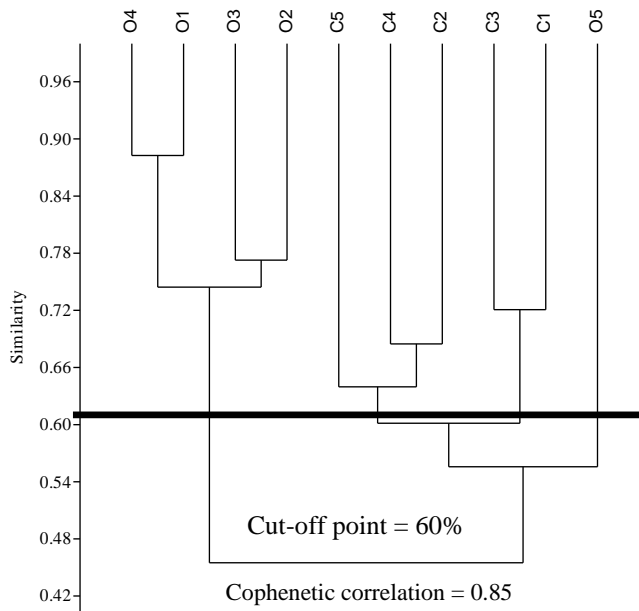
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Figure 4. Correspondence Analyses. Relation between sample spot in each rice crop development stages (V= vegetative; R= reproductive) in each point of the areas with organic management (O) and conventional (C) and parasitoids families in the second season (2014/2015).



641 Figure 5. Cluster Analysis of similarity (Bray-Curtis Index). Relation between abundance
 642 distribution in each family and sample spot in both areas (O.M. and C.M.) in first
 643 season (2013/2014).
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645 Figure 6. Cluster Analysis of similarity (Bray-Curtis Index). Relation between abundance
 646 distribution in each family and sample spot in both areas (O.M. and C.M.) in
 647 second season (2014/2015).
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4 ARTIGO 2

Platygastridae diversity in rice crops with organic and conventional management in southern Brazil*

* Artigo formatado conforme as normas da Neotropical Entomology.

1 Section “Biological Control”
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Platygastridae diversity in rice crops with organic and conventional management in southern Brazil

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Running title: Platygastrids wasps in rice crops

49 **ABSTRACT** - Platygasteridae parasitoids have been referred as important biological control
50 agents in several agricultural systems, mainly to rice crop. Thus, we studied the richness,
51 abundance and populational fluctuation of these Hymenoptera parasitoids through distance
52 gradients of vegetation near cultivated areas under different management of the rice crop
53 (organic and conventional) and under two development stages of plant. The work took place in
54 two irrigated rice crops, one with organic management (O.M.) and another with conventional
55 management (C.M.), in the municipality of Nova Santa Rita, RS, Brazil, during the 2013/2014
56 and 2014/2015 seasons. The parasitoids were collected with Malaise trap arranged at different
57 distances in relation to the native vegetation surrounding the rice crop in both places. Specimens
58 were collected twice a month from seeding until the rice harvest. The most abundant genera
59 were *Idris*, *Telenomus* and *Baeus*. *Telenomus podisi* Ashmead, *Telenomus* sp.3, *Telenomus* sp.2
60 and *Telenomus* sp.1, were the most abundant morphospecies, respectively, and their abundances
61 changing over the seasons. The rice phenological stage influenced the diversity over the two
62 seasons and, in the second, possibly the pesticides use had a negative pressure upon parasitoids
63 assemblage in conventional management. The high abundance of this family indicates that the
64 natural biological control can be occurring in the rice crop, mainly in the organic management.

65

66 **Keywords:** Conservative biological control, egg parasitoids, irrigated rice, native vegetation

67

68 **Introduction**

69 Natural enemies like parasitoids play a key role on agriculture management decreasing pests
70 population density and, consequently, the damage to them (Heinrichs & Barrion 2004). Among
71 them, Platygasteridae (Hymenoptera: Scelioninae, Teleasinae and Telenominae) have been
72 referred as important biological control agents in several agricultural systems, such as

73 *Telenomus* and *Trissolcus* in soybean crop and horticultural species (Santos 2008, Favetti *et al*
74 2013).

75 Parasitoids eggs have high specificity in relation to the hosts (Hanson & Gauld 2006).
76 This is a desirable feature in natural enemies, especially for an applied biological control (Van
77 Driesche & Bellows 1996). Some species have been used for this purpose, such as *Telenomus*
78 *podisi* Ashmead and *Trissolcus basalis* (Wollaston) (Corrêa-Ferreira & Moscardi 1996, Corrêa-
79 Ferreira 2002, Ehler 2002). In Brazil, Corrêa-Ferreira & Moscardi (1996) showed that a
80 inoculative release can control stink bugs populations. It has been used in southern Brazil in
81 soybean (Corrêa-Ferreira 2002).

82 In rice crop (*Oryza sativa* L.), which is important because it can be grown in small,
83 medium and large areas (SOSBAI, 2014), platygastoids belonging to Telenominae are
84 associated mainly to Hemiptera (Ehler 2002, Bambaradeniya & Edirisinghe 2008, Idalgo *et al*
85 2013, Rajmohana & Nisha 2013). In Asian, the black rice bug *Scotinophara* sp. (Hemiptera:
86 Pentatomidae), from India, is parasitized by many platygastroids species such as *Telenomus*
87 *triptus* Nixon, *Telenomus cyrus* Nixon, *Telenomus chloropus* (Thomson), *Telenomus gifuensis*
88 Ashmead (Polaszek & Rajmohana 2007) and *Telenomus oryzae* Rajmohana & Nisha
89 (Rajmohana & Nisha 2013). This parasitoid family always appears with high number of
90 individuals in researches with rice crop diversity, as for example in India, where 40 parasitoids
91 species were found and being 29 of Platygastriidae (Gnanakumar *et al* 2012).

92 Some researches in Brazil show the importance of Platygastriidae in rice fields through
93 natural or conservative biological control, as a controller of pests abundance (Fritz *et al* 2008,
94 Fritz *et al* 2011, Idalgo *et al* 2013, Da Silva *et al* 2016). *Trissolcus basalis* and *T. podisi* are the
95 parasitoids which show highest efficiency in parasitism on *Nezara viridula* (Linnaeus) and
96 *Euschistus heros* (Fabricius) eggs (Ferreira 2003).

97 Thus, the aims of this research were to evaluate the richness, abundance and populational
98 fluctuation of Platygastriidae (Scelioninae, Teleasinae and Telenominae) through distance
99 gradients of preserved vegetation near cultivated areas under different management of the
100 irrigated rice crop (organic and conventional) and under two development stages of rice.

101

102 **Material and Methods**

103 *Study area*

104 The study was carried out in the municipality of Nova Santa Rita, RS, in the metropolitan region
105 of Porto Alegre in the phytogeographic region of the Jacuí watershed, during the 2013/2014 and
106 2014/2015 seasons. In this region, many native trees species occur such as ‘maricá’ (*Mimosa*
107 *bimucronata* (DC) Kuntze), ‘corticeira’ (*Erythrina falcata* Benth.), fig tree (*Ficus luschnatiana*
108 (Miq.) Miq.), ‘angico’ (*Parapiptadenia rigida* (Benth.) Brenan), Surinam cherry (*Eugenia*
109 *uniflora* L.), ‘branquilha’ (*Sebastiania commersoniana* (Baill.) L.B. Sm. & Downs), and
110 ‘açoita-cavalo’ (*Luehea divaricata* Mart. & Zucc.) and many others (COPTEC, 2010). The
111 vegetation near to irrigated rice area is a riparian forest.

112

113 *Organic rice crop*

114 The irrigated rice area with organic management (O.M.) is part of the Capela Settlement
115 (Landless Rural Workers Movement Settlement) in Nova Santa Rita. It has a total area of
116 2169.37 hectares (INCRA, 2007), of which 580 ha belong to COOPAN (Agricultural
117 Production Cooperative Nova Santa Rita). Of these, 220 continuous hectares are allocated for
118 organic rice, which is certified by the Ecological Market Institute (‘Instituto de Mercado
119 Ecológico’ - IMO) (Lanner, 2011). In the settlement an area of 273.52 hectares (13.68% of the

120 total) is designated as a permanent preserve (native forest). In the organic rice field, the levees
121 are maintained with wild vegetation.

122 The Capela Settlement is located southwest of the Caí River. To the north, in a range of
123 417 m, it borders the Mineiro stream, which is 7m wide on average. It is also watered by smaller
124 streams, but the main water resource is the Padre weir, which occupies 67 hectares of the
125 property and has a dam of 300 m length. Previous wetland areas have been exploited for rice-
126 growing activity (Fagundes and Gastal, 2008). There are 100 families settled in the region, with
127 an average area of 21.69 hectares per lot (INCRA, 2007).

128 Data samplings were made in different rice lots at each season.

129 In the first season (December, 2013 to April, 2014), five points were defined for the
130 installation of traps centered around this coordinate 29° 47' 16.62" S and 51° 21' 00.91" W. In
131 the area where the traps were installed, it was used the pre-germinated planting system with
132 cultivar EPAGRI 108. It has late cycle, lodging resistance, indirect iron toxicity, moderately
133 resistant to panicle blast, stands out for its excellent grain quality and high reproductive potential
134 (SOSBAI, 2014).

135 In view of the difficult access to the area in the first year, another five points were defined
136 in the second year (November, 2014 to March, 2015) for the installation of traps centered around
137 this coordinate 29° 46' 48.90" S, 51° 21' 48.38" W. The management of the area was the same
138 as in the first year.

139 In the first year, the traps were separated 100 m from each other, in a distance gradient
140 starting from inside the forest to the middle of the crop, in a straight line (Fig 1). In the second
141 year, the distance was extended to 200 m. Each trap site was named by the location of the
142 Malaise trap, being O5 (Spot 5 - inside the forest), O4 (Spot 4), O3 (Spot 3), O2 (Spot 2), O1
143 (Spot 1 - greatest distance from forest) (Fig 1).

144 *Conventional rice crop*

145 The irrigated rice area with conventional management (C.M.) is located in the ‘Gaúcha’ Farm,
146 a private property of 300 hectares, bordered to the southwest by the Caí River. Tillage is the
147 planting system, and the cultivar used was IRGA428. It has a medium cycle, high productivity
148 potential, tolerance to Only and Kifix herbicides, tolerance to toxicity from excess iron, is
149 moderately susceptible to leaf blast and susceptible to blast on panicle (SOSBAI, 2014).

150 The conventional management adopted by the producer follows the IRGA
151 recommendations, with the use of chemical inputs and application of agricultural pesticides
152 according to the annual manuals for rice management (SOSBAI, 2012). For insect collecting,
153 the experimental design was the same of the organic management area (Fig 1), with the points
154 set between 29° 46’ 16.65” S and 51° 21’ 43.31” W. This area is approximately 2.5 km distant
155 from the organic management area. The area for trap installation was the same in both seasons.
156 In the conventional rice field, the levees are maintained without wild vegetation by chemicals
157 spraying.

158 As in the O.M. area, in the first year (2013/2014), the traps were from each other by 100
159 m, in a straight line, and in the second (2014/2015), the distance was extended to 200 m. Each
160 spot also was named by the location of Malaise trap, being C5 (Spot 5), C4 (Spot 4), C3 (Spot
161 3), C2 (Spot 2), C1 (Spot 1).

162 In the first season analyzed regulators insecticides were used (buprofezin and
163 benzylurea) and in the second season were used non-selective (neurotoxic) insecticides
164 (neonicotinoids and pyrethroids) (farmer personal communication Mr. Denis).

165 *Platygastridae sampling and identification*

166 For capturing the parasitoids five Malaise traps (Townes 1972) were placed in the levees of each
167 of the areas (Fig 1). The collection method was improved, by putting PVC pipes cut in half to

168 simulate a “gutter” in each trap, so that insects which have not reached the vials could drop in
169 the “gutter” (containing water and detergent) located on the ground, comprising the entire length
170 of the trap (Gullan & Cranston, 2008).

171 Samples were collected every two weeks during two rice seasons, 2013/2014 and
172 2014/2015, from seeding to harvest. The traps remained mounted in the points for 24 hours and
173 thereafter the vials with the collected insects were taken. These were transferred to 70% alcohol,
174 labeled with the collection site and transported to the Laboratory for Biological Control of
175 Insects (CBLAB) of UFRGS. In the laboratory, the samples were screened using a
176 stereomicroscope Nikon SMZ445, selecting the Platygastriidae (Scelioninae, Teleasinae and
177 Telenominae) hymenopteran parasitoids. The specimens were mounted on entomological pins
178 with double mounting and placed in a reference collection. After, identified in specific level
179 through Keys (Johnson 1984, Johnson 1987, Johnson & Musetti 2011, Masner 1976, Masner
180 1980, Masner 1983, Muesebeck 1977) and compared with the specimens on C.A. Triplehorn
181 Insect Collection, Ohio State University, Columbus, OH.

182 .

183 *Statistical analyses*

184 Considering the extent of the two areas and their characteristics, it was impossible to make
185 replicas of the samples, so that evaluations were considered exploratory.

186 The rice development stages were identified according to Counce *et al* (2000) into
187 vegetative and reproductive stages. Here we named indicated O.V or C.V (organic or
188 conventional vegetative stage of rice) and O.R or C.R (organic or conventional reproductive
189 stage), respectively.

190 Fluctuations in the abundance of individuals of the most abundant species over the
191 samples and rice management in both season were evaluated.

192 Rarefaction curves were constructed to compare richness between the both places of rice
193 crop management (O.M. and C.M.) through the PAST Program (Hammer *et al* 2001). Estimated
194 richness was calculated for each study site via the Chao 1 and Bootstrap estimates, using
195 EstimateS software, version 9.1.0 (Colwell 2013).

196 Correspondence Analysis (CA) and Bray-Curtis Cluster Analysis plots were built
197 through the PAST program (Hammer et al., 2001). Plots are constructed with the main
198 components of lines and columns allowing visualization of the relationship between groups,
199 where the proximity of the points relating to the line (Scelioninae, Teleasinae and Telenominae
200 genera) and column (spots and rice stages) indicates association and the distance indicates
201 repulsion (Greenacre and Hastie, 1987). The Cluster Analysis groups the data in a dendrogram,
202 in which the level of similarity (or dissimilarity) is indicated on the vertical scale. On the
203 horizontal axis, the sample elements are reported in a convenient order for the clustering
204 (Mingoti, 2005).

205

206 **Results**

207 *Faunistic data*

208 Among the collected genera in both seasons, it highlights for *Idris* spp. (11 morphospecies),
209 *Telenomus* spp. (8 morphospecies) and *Baeus* spp. (5 morphospecies) for the higher richness.
210 Several genera have the first record for Rio Grande do Sul, Brazil (Table 1).

211

212 *First season*

213 In the first season, 268 individuals were collected in the rice area with organic management
214 (O.M), distributed in 31 morphospecies and 172 individuals were collected in the rice area with
215 conventional management (C.M.), distributed in 24 morphospecies.

216 There was variation between species in each area and development stages of rice crop
 217 (Table 1). *Telenomus podisi* was the most abundant in both areas with 31.30% in O.M and
 218 38.4% in C.M, and *Telenomus* sp.3 the second most abundant with 21.03% (O.M.) and 15.1%
 219 (C.M.).

220 The abundance of these species varied over the season (Fig 2). In both areas, *T. podisi*
 221 and *Telenomus* sp.3 were more abundant in the reproductive stage than in the vegetative.

222

223 *Second season*

224 In the second season, 151 individuals were collected in the rice area with organic management
 225 (O.M.), distributed in 34 morphospecies and 93 individuals were collected in the rice area with
 226 conventional management (C.M.), distributed in 21 morphospecies.

227 *Telenomus podisi* was the most abundant in both areas with 23.8% in O.M. and 49.5%
 228 in C.M. The second most abundant in O.M. area was *Telenomus* sp.2 (9.3%) and in C.M.,
 229 *Telenomus* sp.1 (18.3%) (Table 1).

230 In both areas, *T. podisi* was more abundant in the reproductive stage than vegetative (Fig
 231 3). *Telenomus* sp.1 had higher abundance in the vegetative stage of C.M. and *Telenomus* sp.2
 232 was most abundant in the reproductive stage of O.M.

233

234 *Association between platygastroids genera, sampling spots, management, and phenological* 235 *stages*

236 Through correspondence analysis, the points were represented on two axes, indicating that the
 237 association between lines (Scelioninae, Teleasinae and Telenominae genera) and columns (spots
 238 and rice stages) is two-dimensional, 50% of the relationship being explained in these two axes
 239 in season 1 and 44% of the relationship explained in season 2.

240 In the first season, samples are mixed on both sides of the x and y axes, showing similar
241 composition between areas (Fig 4). Some genera, such as *Telenomus*, *Trissolcus*, *Macroteleia*
242 and *Triteleia* showed relationships with certain sampling points and phenological periods of the
243 plant CR and OR. On the other hand, *Xenomerus* and *Baryconus* genera appear to be distributed
244 on distinct sides far away from the axes and had no connexion with sampling points. The points
245 organic 5 (O5) and conventional 5 (C5) were the most dissimilar from the others.

246 In the second season (Fig 5), the genera showed dissimilarities between them, being
247 more dispersed on chart than season before. *Telenomus* spp. and *Macroteleia* spp. had the same
248 similarity with reproductive stages of O.M. and C.M. In the other hand, *Anteris* and
249 *Probaryconus* genera had not relationship with any sampling points. The points O5, C3 and C5
250 were the most dissimilar from the others.

251 Regarding all sampling (assembling both seasons), differences between species
252 composition and abundance are recognized among phenological stages in both season through
253 cluster analysis. The high cophenetic correlation (0.83) confirms the adjustment of data to the
254 Bray-Curtis model (Fig 6).

255 There was similarity among phenological stages, independent of rice management.
256 Thereby, in the first season, the diversity similarity was influenced by crop phenological stages
257 (65% in vegetative and 64% in reproductive) and was not by management. In the second season,
258 however, it was not visible.

259

260 *Species richness*

261 The species accumulation curves (S) at each area indicates that richness was not fully sampled,
262 as illustrated by the ascending curves (Fig 7). The richness sampled to O.M. was 45
263 morphospecies and, to conventional, 32 morphospecies. The variables to estimate areas richness

264 (Magurran 2011), in O.M. there were 9 singletons, 12 doubletons, 13 unicates and 12 duplicates
265 and in C.M. there were 12 singletons, 5 doubletons, 14 unicates and 6 duplicates.

266 The rarefaction curve shows differences between the two areas for Scelionidae diversity
267 (Fig 8), because in the cut-off point (around 250 individuals) the observed diversity in the
268 smaller community (C.M.) did not reach the 95% confidence interval of the larger community
269 (O.M.).

270

271 **Discussion**

272 Among the genera with the highest species richness, *Idris* and *Baeus* belong to the Baeini tribe
273 and are known for parasite spiders eggs (Stevens & Austin 2007, Valerio *et al* 2013). Spiders
274 are frequent in rice crops, so several species from this group were expect (Bambaradeniya &
275 Edirisinghe 2008, Fritz *et al* 2011). The noteworthy abundance of *Telenomus* species indicates
276 them importance on local trophic chain, acting in biological control of pests or others
277 herbivorous on the crop and adjacent areas. The host specificity is an important feature and
278 makes them effective on biological control (Hanson & Gauld 2006). We found it in higher or
279 lower degree within some *Telenomus* group (Johnson 1984), for example, *Telenomus arzamae*
280 Riley, *Telenomus californicus* Ashmead and *Telenomus dalmanni* (Ratzeburg) only attack
281 Lepidoptera; *Telenomus crassiclava* Nixon parasitize Fulgoridae; *Telenomus floridanus*
282 (Ashmead), Lygaeidae; *Telenomus laricis* Walker, Miridae; *Telenomus nigrocoxalis* Ashmead,
283 Rhopalidae; *T. podisi*, Pentatomoidea and *Telenomus tabanivorus* (Ashmead), Diptera
284 (Tabanidae, Asilidae, Stratiomyidae) (Hanson & Gauld 2006). Therefore, when to determine
285 the *Telenomus* species will be possible infer the host association.

286 Also, several species of *Telenomus* spp. were collected in rice crop in Philippines in a
287 research about trophic interactions (Schoenly *et al* 1996). Others species are associated to rice

288 pests, such as *Telenomus nr. triptus* Nixon and *Telenomus rowani* (Gahan, 1925) collected in
289 rice crops in SriLanka at crop and rice levees (Bambaradeniya & Edirisinghe 2008). In India, *T.*
290 *rowani* was the parasitoid emerged from yellow stem borer *Scirpophaga incertulas* (Walker)
291 (Chakraborty *et al* 2016).

292 *Telenomus podisi*, the most abundant specie in both areas and seasons, is known for
293 parasitize Pentatomidae eggs in many cops. In rice fields in Brazil, pentatomids are pests that
294 damage the plant stems and grain, such as rice stem bug *Tibraca limbativentris* Stål and the
295 small rice stink bug *Oebalus poecilus* (Dallas) (Ferreira & Barigossi 2001, Idalgo & Sant'Ana
296 2010, Machado & Garcia 2010). A parasitism rate of 23.4% was found in *T. limbativentris* in
297 Maranhão with *T. podisi* being more frequent (Maciel *et al* 2007). In rice crops in Santa
298 Catarina, a parasitism rate of 70% was found with the same species in rice stem bug (Riffel
299 2007). In Rio Grande do Sul, *T. podisi* had a parasitism rate of 75% over *T. limbativentris*
300 (Idalgo *et al* 2013). Regarding to small rice stink bug, *Oebalus* spp. eggs are parasitized by
301 *Telenomus mormidae* Lima (Ferreira & Barrigossi 2004, Panizzi 2008).

302 *Trissolcus*, also present in this survey, together with *Telenomus* spp., are considered the
303 platygastroids genera more important for Pentatomidae biological control (Hanson & Gauld
304 2006).

305 Other genera from this family also have hosts insects associated to rice fields (Schoenly
306 *et al* 1996, Gangurde 2007, Fritz *et al* 2011). For example, *Calliscelio* spp. (Masner *et al* 2009),
307 *Cremastobaeus* spp. (Loiacono & Mulvany 1987, Masner & Hanson 2006), *Duta* spp. (Masner
308 1991, Masner & Hanson 2006), *Paridris* spp. (Talamas *et al* 2012) that parasite Gryllidae eggs.
309 *Calotelea* species was registered in Orthoptera and Odonata eggs in rice fields (Kononova &
310 Fursov 2007) and *Triteleia* parasitize Orthoptera (Popovici *et al* 2011).

311 Regarding to phenological stages of crop, *T. podisi* (O.M. and C.M.), *Telenomus* sp.2
312 (O.M.) and *Telenomus* sp.3 (O.M. and C.M.) were most abundant in reproductive stages, while
313 *Telenomus* sp.1 (C.M.) and *Trissolcus* spp. (O.M. and C.M.) were most abundant in vegetative
314 stage. The specific difference can be associated to pentatomids pests that cause damage in the
315 stem and grain, such as rice stem bug and the small rice stink bug (Ferreira & Barigossi 2001,
316 Idalgo & Sant'Ana 2010, Machado & Garcia 2010). These pests are more abundant in
317 reproductive stage (SOSBAI 2014). In southern Brazil, a higher richness of Platygastriidae in
318 vegetative stage in rice crop was found (Simões-Pires *et al* 2016). On the other hand, according
319 with our research, Bambaradeniya & Edirisinghe (2008) in SriLanka, found more diversity in
320 the reproductive stage. Arthropod diversity variation (phytophagous and natural enemies) had
321 relationship with rice phenological stage (75% correlation coefficient) and parasitoid density
322 had high positive correlation with pest density, therefore, phytophagous as well as parasitoids,
323 have association to crop phenological stage (Bambaradeniya & Edirisinghe 2008).

324 The points O5 and C5 were the most dissimilar in both season, through correspondence
325 analysis. These points were inside native vegetation (preservation area) and can be the reason
326 of the difference with the others on the crop.

327 Some genera had relationship with one of these points, such as *Phanuromyia* and *Scelio*
328 (O5) in the first season and *Trimorus* (O5), in the second season. *Phanuromyia* is a genus known
329 for parasitize Fulgoridae and Issidae eggs (Johnson & Musetti 2003), *Scelio* for parasitizing
330 Acrididae (Yoder *et al* 2009, Yoder *et al* 2014) and *Trimorus*, Carabidae (Masner & Hanson
331 2006). *Anteris* and *Probarryconus* were the most dissimilar genera, showing a relationship with
332 conventional points C3 and C5 in the second season. Both genera have records in egg parasitism
333 of Orthoptera, mainly in Grylloidea (Gryllidae) (Masner & Hanson 2006). There are records of

334 sub-aquatic Grylloidea, Phalangopsidae, in Neotropical region (Sperber *et al* 2003), justifying
335 their presence in some sampling points.

336 In the first season, *Baryconus* and *Xenomerus* were further away from all sampling
337 points, probably because their lower abundance and association with some point in organic
338 management. *Baryconus* spp. are known for parasitize Tettigoniidae (Muesebeck 1977, Ritchie
339 & Masner 1983, Masner & Hanson 2006), while *Xenomerus* have unknown hosts.

340 Besides *Telenomus* and *Idris* genera cited above, *Macroteleia* and *Gryon* showed
341 similarity with several sampling points of both management and seasons. *Macroteleia* spp. are
342 known to parasitize Tettigoniidae (Masner & Hanson 2006, Chen *et al* 2013) and *Gryon* spp.
343 are known to parasitize Heteroptera eggs (Masner & Hanson 2006).

344 The similarity influenced by crop phenological stage can appears on cluster analysis,
345 that reached up to 65% in the first season. Both areas shared the same vegetation profile, i.e. the
346 preserved areas near to the crop have similar faunistic composition (COPTec 2010), so
347 probably the parasitoids assemblage in theses refuges areas are alike. Thus, platygastroids
348 assemblage composition in the rice fields was more influenced by rice phenological stage, which
349 attracted different hosts and, consequently, different parasitoids were attracted to the local.

350 In the second season, there was no similarity found and the management may have had
351 a greater influence on composition. In the first season, in conventional management growth
352 regulating insecticides were used (Buprofezin and Benzoylurea), which are more selective to
353 natural enemies; in the second, however, the farmer used neurotoxic products (Neonicotinoids
354 and Pyrethroids) (personal communication, Mr. Denis), not selective to natural enemies.

355 Therefore, in the first season, the management did not have a large effect in
356 Platygastriidae assemblage composition, being more influenced by adjacent vegetation and crop

357 development phases. While in the second season, a more harmful human intervention can have
358 affected the assemblages in conventional management.

359 The species accumulation curves (S) at each area indicates that richness was not fully
360 sampled, since are on the rise on the line graph. Invertebrate diversity is still poorly documented
361 and assemblages are not closed systems, thus, the cumulative species list must rise indefinitely
362 when new colonizers arrive. For Magurran (2011), the best thing we can reach is an estimate of
363 species richness and they are based on the numbers of rare species (singletons and doubletons),
364 their representativeness throughout the sample period (constant species or accessories - unicates
365 and duplicates) and their proportion regarding to richness and abundance of total sampling in
366 each assemblage. Both areas have a high number of rare species (47% and 53% in organic
367 management and conventional, respectively), therefore it was expected a high richness pointed
368 out by estimators.

369 Chao 1 estimated that 94.22% of richness in O.M. and 69.03% in C.M. was sampled.
370 Chao 1 is a simple estimator of absolute number of species in an assemblage (Chao 1984), being
371 based on rare species number (Magurran 2011). Therefore, it is the best for estimating the
372 minimum number of species in an assemblage (Gotelli & Colwell 2011) and a good estimator
373 to evaluate our data.

374 In O.M. the doubletons numbers were larger than singletons, hence the high index
375 percentage, while in C.M., the opposite was observed, resulting in a lower index.

376 The Bootstrap estimator requires incidence data (Smith & Van Belle 1984) and differs
377 from the others by use data from all species collected to estimate the total richness, using the
378 unicates and duplicates numbers (Colwell 2013) and not only rare species; therefore, it is
379 generally the lowest value estimator. According to it, 87.90% of richness from O.M. and 84.49%
380 of richness from C.M. was sampled.

381 In our analysis, we choose to evaluate the richness with more than one estimator because
382 rarity levels are exaggerated in researches with insects assemblages and when a single sampling
383 technique is employed, being a factor that causes the estimators to fail (Longuino *et al* 2002).

384 Thus, through the richness estimators, the areas had different diversity patterns. By
385 applying simultaneously, the curves of accumulation of species and Chao 1, it is possible a
386 significant view of species diversity and estimate of total richness (Magurran 2011).

387 Rarefaction curves of both areas corroborate estimators data and show differences in
388 species diversity among them, because the observed diversity in C.M. in cut-off point is not
389 within 95% confidence interval of rarefaction curve of O.M., which is the larger community.

390 The greatest diversity of parasitoids in O.M. in both seasons must be due to the difference
391 on crop management without agrochemicals, reducing the impacts upon the insects. Decrease
392 on phytophagous, predators and parasitoids diversity after agrochemicals application is well
393 known. Besides of, a faster increase in pests than in natural enemies population after the
394 pesticide effect, called pests resurgence, have been emphasized by many researchers (Pingali &
395 Gerpacio 1997, Tanaka *et al* 2000, Gangurde 2007; Bambaradeniya & Edirisinghe 2008).

396 For final considerations, the rice phenological stage influenced the diversity over the
397 two seasons with morphospecies and abundances different in both stages. It must be related to
398 different hosts, oftentimes phytophagous insects, in each crop development. Investigations
399 related to it can bring important information about the dynamics and relationships of these
400 populations in the rice system. However, in the second season, possibly the pesticides use had
401 a negative pressure upon parasitoids assemblage in conventional management. The chemical
402 insecticides in rice crops can change parasitoids richness and abundance through changes of
403 selective insecticides to natural enemies (growth regulators) to non-selective insecticides
404 (neurotoxic).

405 The records of this work highlight the importance of the Platygastriidae for the biological
 406 control of pests in rice fields. The high abundance of this family indicates that the natural
 407 biological control can be occurring in the rice crop, mainly in the organic management. Several
 408 genera have the first record for Rio Grande do Sul, Brazil.

409

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 658
- 659 Table 1 Morphospecies of Platygasteridae (Scelioninae, Teleasinae and Telenominae) sampled
 660 in the first (2013/2014) and second crop season (2014/2015) over vegetative and reproductive
 661 stage of rice crop in organic management area (O.M.) and conventional management area
 662 (C.M.), Nova Santa Rita, RS.
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	1° Season						2° Season					
	O.M			C.M			O.M			C.M		
	Veg	Rep	Fr (%)	Veg	Rep	Fr (%)	Veg	Rep	Fr (%)	Veg	Rep	Fr (%)
Platygasteridae												
<i>Anteris</i> sp.1*	0	0	0.0	0	0	0.0	0	0	0.0	2	0	2.2
<i>Baeus</i> sp.1*	0	0	0.0	0	0	0.0	2	1	2.0	1	0	1.1
<i>Baeus</i> sp.2*	3	0	1.1	0	0	0.0	1	0	0.7	0	0	0.0
<i>Baeus</i> sp.3*	0	0	0.0	1	0	0.6	0	1	0.7	0	0	0.0
<i>Baeus</i> sp.4*	0	0	0.0	0	0	0.0	1	2	2.0	0	0	0.0
<i>Baeus</i> sp.5*	0	2	0.8	3	5	4.7	0	0	0.0	1	0	1.1
<i>Baryconus</i> sp.1*	7	1	3.0	0	0	0.0	0	2	1.3	0	1	1.1
<i>Calliscelio</i> sp.1*	5	1	2.2	3	3	3.5	1	0	0.7	0	0	0.0
<i>Calotelea</i> sp.1*	0	0	0.0	2	1	1.7	0	1	0.7	0	0	0.0
<i>Cremastobaeus</i> sp.1*	0	1	0.4	2	0	1.2	0	3	2.0	1	0	1.1
<i>Cremastobaeus</i> sp.2*	0	1	0.4	0	0	0.0	0	6	4.0	0	0	0.0
<i>Duta</i> sp.1*	4	6	3.7	7	0	4.1	0	2	1.3	0	1	1.1
<i>Duta</i> sp.2*	0	1	0.4	0	0	0.0	1	4	2.9	1	0	1.1

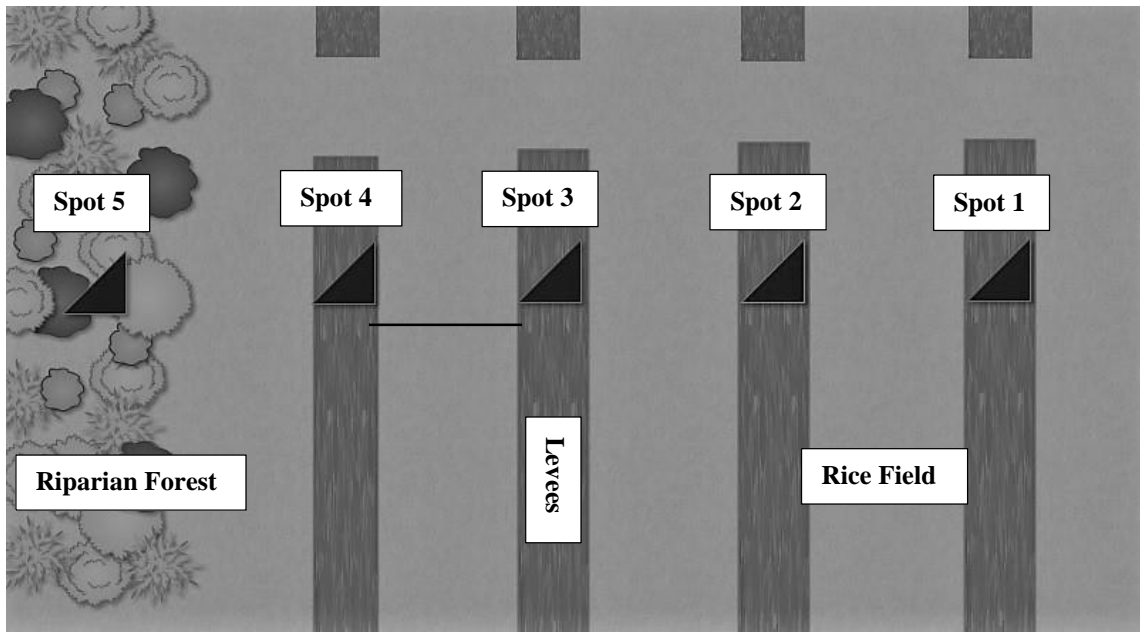
664 Table 1 continue

<i>Eumicrosoma</i> sp.1*	0	0	0.0	0	0	0.0	0	2	1.3	0	0	0.0
<i>Gryon</i> sp.1	1	0	0.4	0	0	0.0	1	6	4.6	2	0	2.2
<i>Gryon</i> sp.2	0	0	0.0	0	0	0.0	2	0	1.3	0	0	0.0
<i>Idris</i> sp.1*	0	0	0.0	0	0	0.0	0	1	0.7	0	0	0.0
<i>Idris</i> sp.2*	0	0	0.0	0	0	0.0	1	1	1.3	0	0	0.0
<i>Idris</i> sp.3*	0	0	0.0	0	0	0.0	0	2	1.3	0	0	0.0
<i>Idris</i> sp.4*	1	0	0.4	0	0	0.0	0	1	0.7	0	0	0.0
<i>Idris</i> sp.5*	0	0	0.0	0	2	1.2	0	0	0.0	0	2	2.2
<i>Idris</i> sp.6*	0	1	0.4	0	0	0.0	0	1	0.7	0	1	1.1
<i>Idris</i> sp.7*	0	0	0.0	0	1	0.6	2	0	1.3	0	0	0.0
<i>Idris</i> sp.8*	1	0	0.4	0	0	0.0	0	1	0.7	0	0	0.0
<i>Idris</i> sp.10*	0	0	0.0	1	1	1.2	0	0	0.0	0	0	0.0
<i>Idris</i> sp.11*	1	0	0.4	1	0	0.6	0	0	0.0	0	0	0.0
<i>Idris</i> sp.12*	0	1	0.4	0	0	0.0	0	0	0.0	0	0	0.0
<i>Macroteleia</i> sp.1*	3	8	4.1	2	4	3.4	0	2	1.3	1	0	1.1
<i>Opisthacantha</i> sp.1*	3	3	2.2	2	1	1.7	4	1	3.3	1	0	1.1
<i>Paridris</i> sp.1*	3	0	1.1	0	0	0.0	1	0	0.7	0	0	0.0
<i>Phanuromyia</i> sp.1*	7	2	3.4	0	1	0.6	0	7	4.6	0	2	2.2
<i>Probaryconus</i> sp.1*	0	0	0.0	0	0	0.0	0	0	0.0	2	0	2.2
<i>Probaryconus</i> sp.2*	0	0	0.0	0	0	0.0	0	0	0.0	0	1	1.1
<i>Scelio</i> sp.1*	1	2	1.1	0	0	0.0	0	0	0.0	0	0	0.0
<i>Trimorus</i> sp.1*	0	1	0.4	0	0	0.0	2	1	2.0	0	0	0.0
<i>Trimorus</i> sp.2*	11	1	4.5	6	1	4.1	1	1	1.3	2	0	2.2
<i>Trissolcus</i> sp.1	0	0	0.0	1	0	0.6	2	0	1.3	0	0	0.0
<i>Trissolcus</i> sp.2	1	0	0.4	2	0	1.2	0	0	0.0	0	0	0.0
<i>Trissolcus</i> sp.3	0	0	0.0	1	0	0.6	0	0	0.0	0	0	0.0
<i>Triteleia</i> sp.1*	0	3	1.1	0	0	0.0	0	0	0.0	0	0	0.0
<i>Telenomus</i> sp.1	8	5	4.9	7	4	6.4	5	7	8.0	14	3	18.3
<i>Telenomus</i> sp.2	9	3	4.5	3	1	2.3	1	13	9.3	1	0	1.1
<i>Telenomus</i> sp.3	12	45	21.3	11	15	15.1	7	5	8.0	5	1	6.5
<i>Telenomus</i> sp.4	0	0	0.0	0	0	0.0	0	4	2.7	0	0	0.0
<i>Telenomus</i> sp.5	5	6	4.1	9	0	5.2	0	2	1.3	0	1	1.1
<i>Telenomus</i> sp.6	31	53	31.3	22	44	38.4	11	25	23.8	15	31	49.5
<i>Telenomus</i> sp.7	1	0	0.4	1	0	0.6	0	0	0.0	0	0	0.0
<i>Telenomus</i> sp.8	2	0	0.8	1	0	0.6	0	0	0.0	0	0	0.0
<i>Xenomerus</i> sp.1*	1	0	0.4	0	0	0.0	0	0	0.0	0	0	0.0
Total	121	147	100.0	88	84	99.9	46	105	99.9	49	44	99.9

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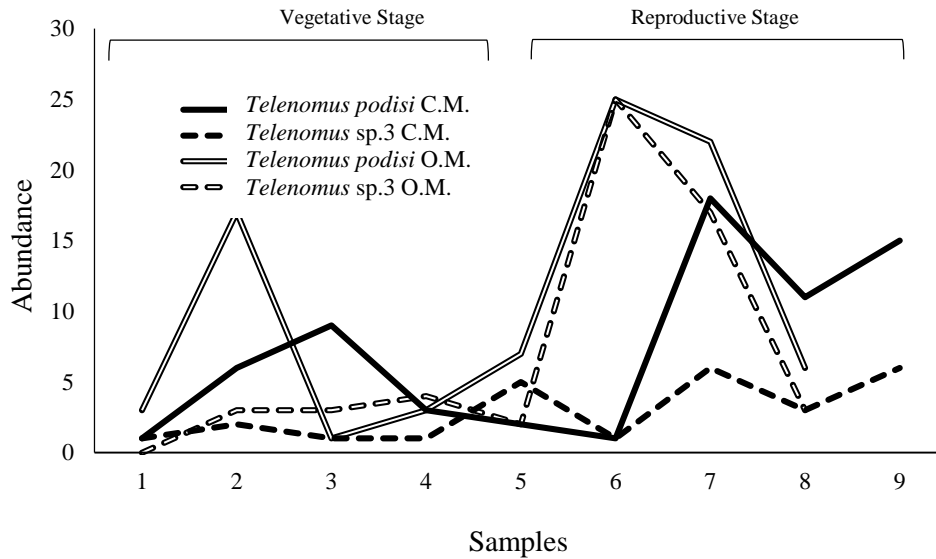
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*Genera with first record for Rio Grande do Sul, Brazil.



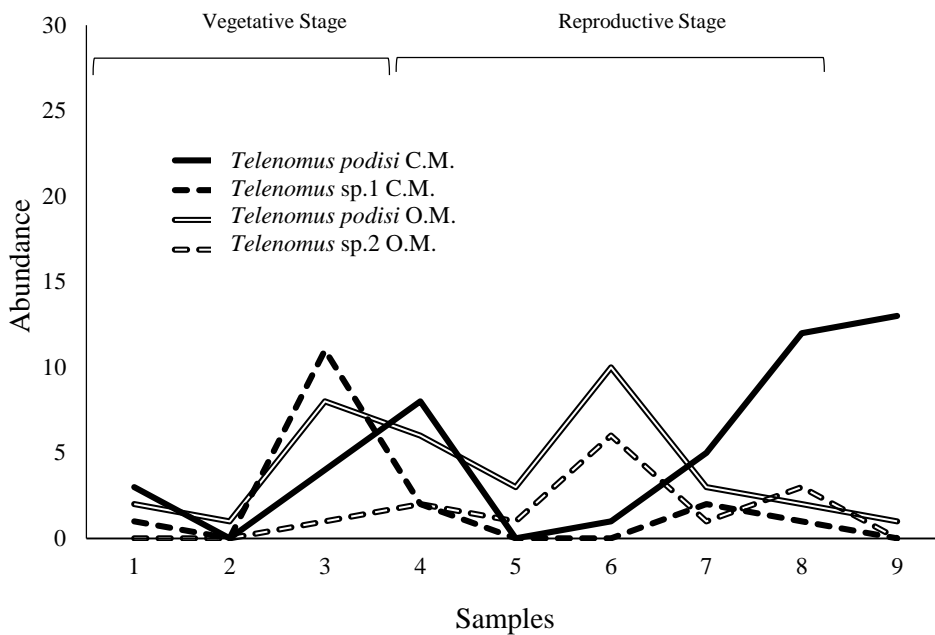
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Fig 1 Layout showing Malaise traps (▲) position on rice field and riparian forest in both areas, Organic Management Area (O.M.) and Conventional Management Area (C.M.), in Nova Santa Rita, RS. (—) Distance between traps (100m first season; 200m second season).



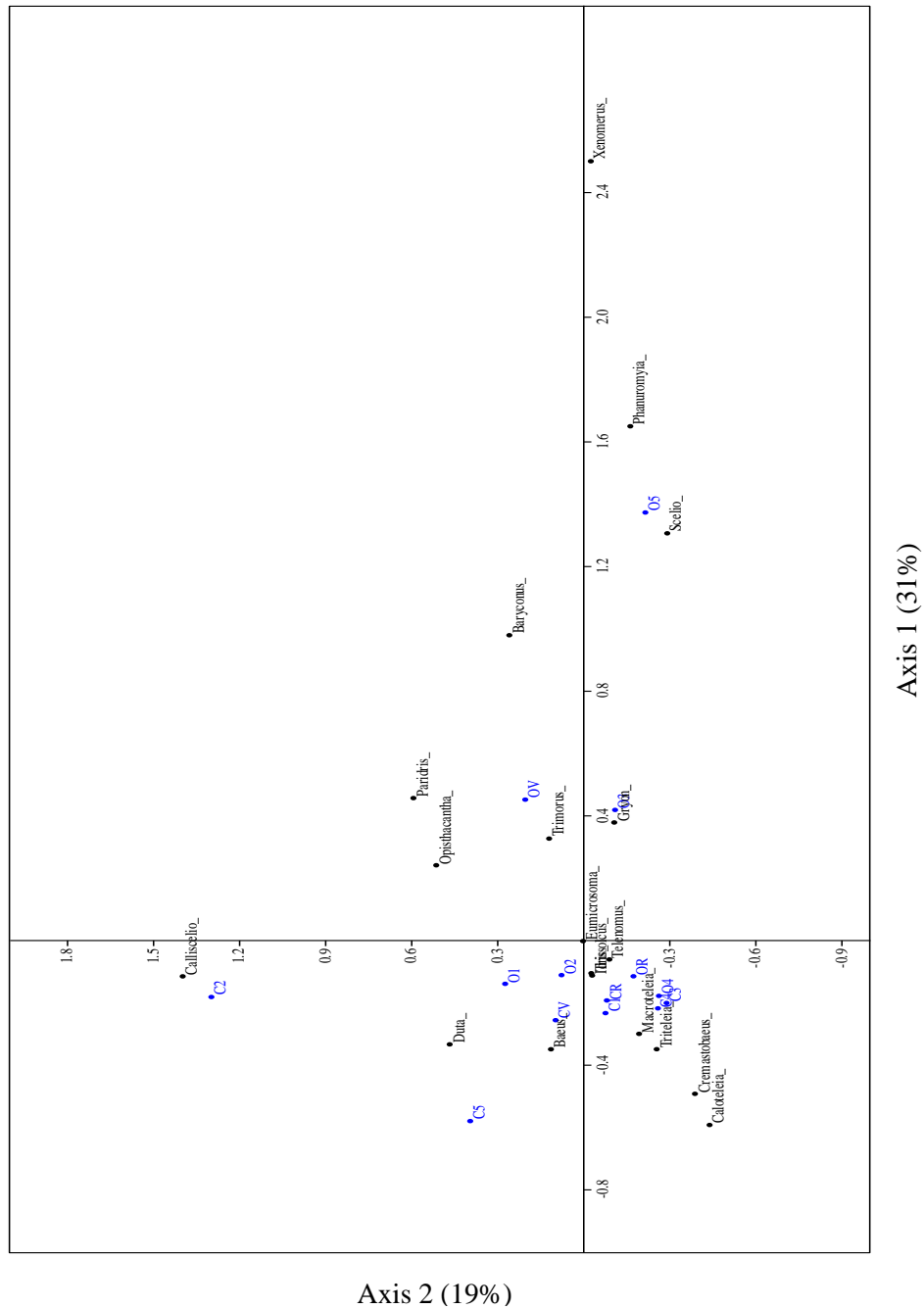
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Fig 2 Fluctuations in the abundance of individuals of the most abundant species (*Telenomus podisi* and *Telenomus sp.3*) in rice fields with organic management (O.M.) and conventional management (C.M.). Nova Santa Rita, RS, Brazil (December, 2013 to April, 2014).



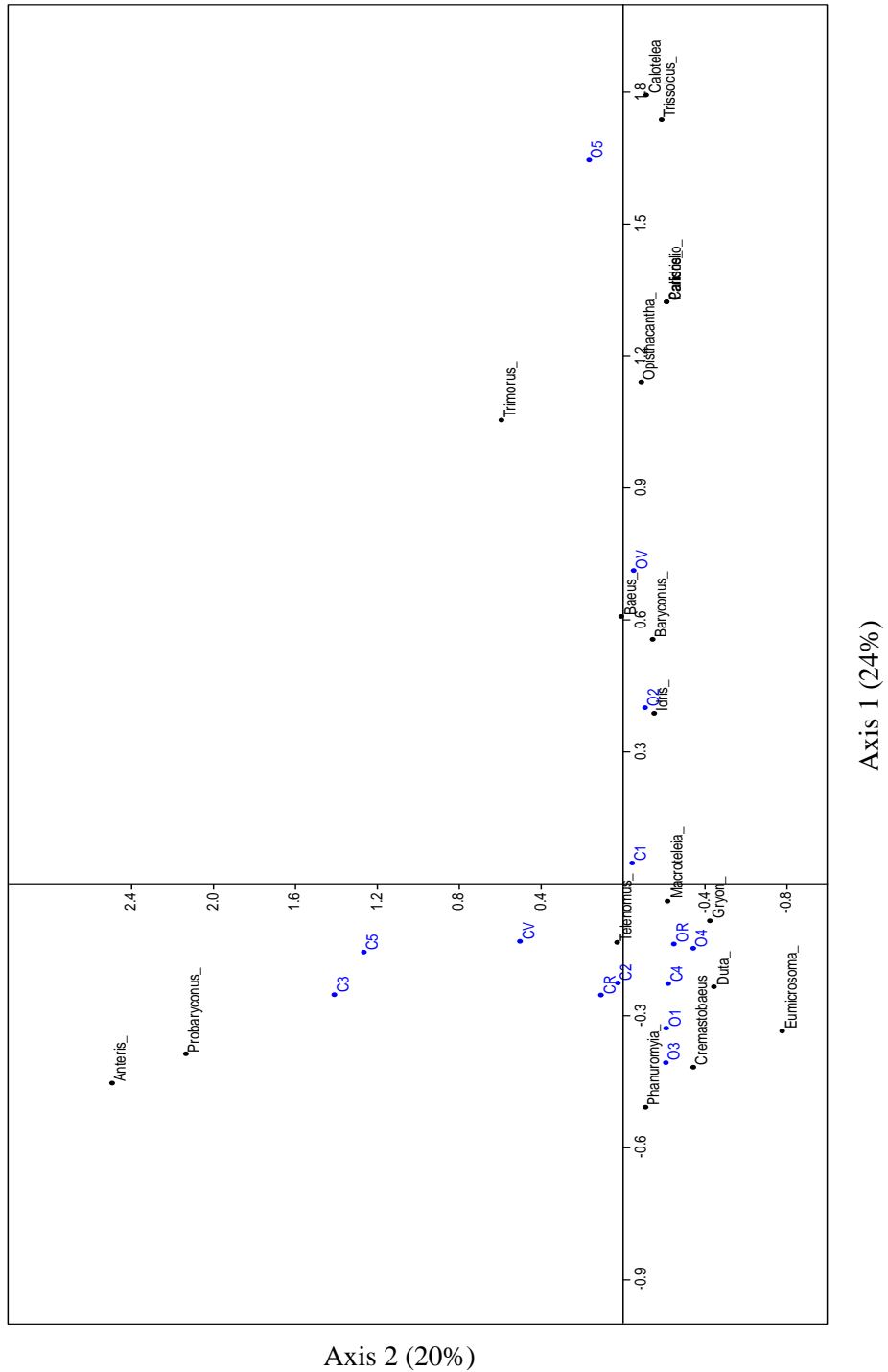
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Fig 3 Fluctuations in the abundance of individuals of the most abundant species (*Telenomus podisi*, *Telenomus sp.1* and *Telenomus sp.2*) in rice fields with organic management (O.M.) and conventional management (C.M.). Nova Santa Rita, RS, Brazil (November, 2014 to March, 2015).



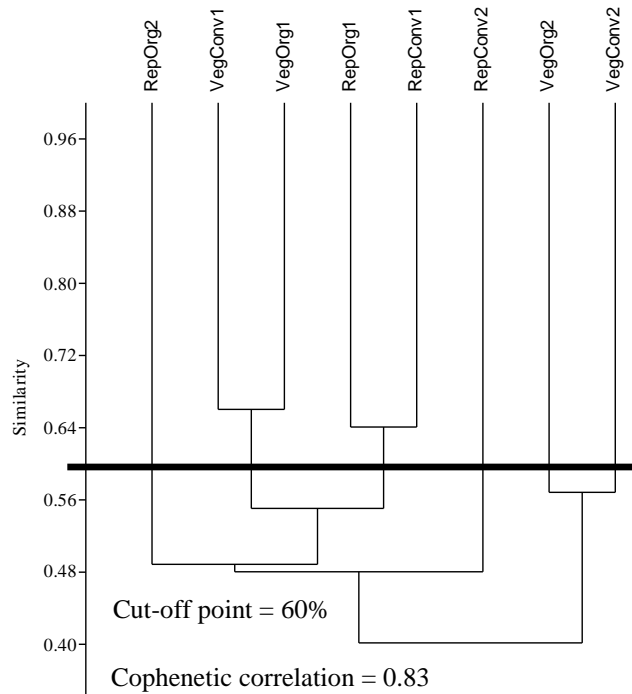
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Correspondence Analyses. Relation between sample spot in each management, rice crop development stages (V= vegetative; R= reproductive) in each point of the areas with organic management (O) and conventional (C) and platygastroids species in the first season (2013/2014), Nova Santa Rita, RS, Brazil.



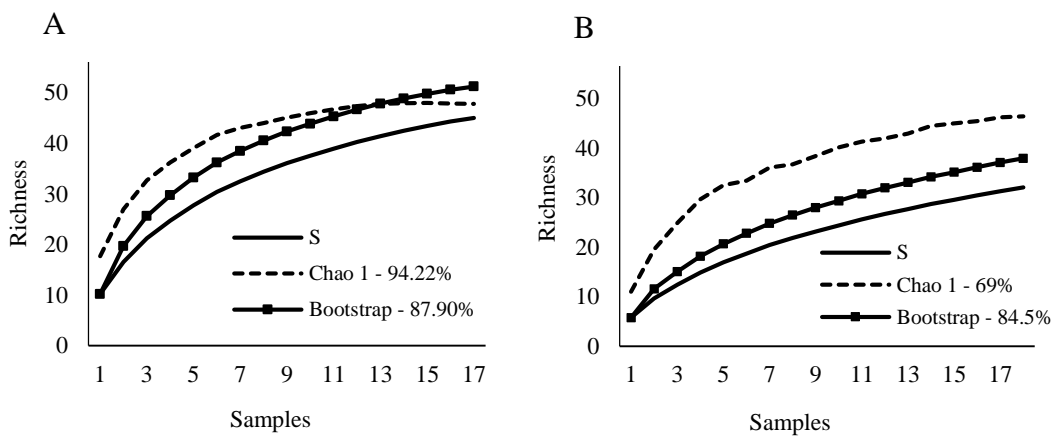
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Correspondence Analyses. Relation between sample spot in each management, rice crop development stages (V= vegetative; R= reproductive) in each point of the areas with organic management (O) and conventional (C) and platygastroids species in the second season (2014/2015), Nova Santa Rita, RS, Brazil.



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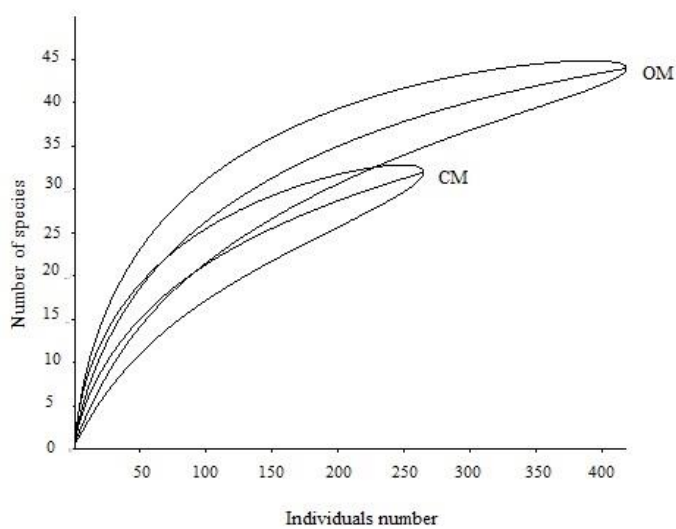
Fig 6 Cluster Analysis of similarity (Bray-Curtis Index). Relation between platygastroids abundance distribution and rice phenological stages vegetative (Veg) and reproductive (Rep) in both areas (organic = Org and conventional = Conv) in first season (2013/2014 = 1) and second season (2014/2015 = 2), Nova Santa Rita, RS, Brazil. Cophenetic correlation = 0.83; Cut-off point = 60%



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Fig 7 Estimated richness curves (% of estimated richness) of platygastroids species to Chao 1 and Bootstrap estimators (randomized 500 times) and accumulation curve (S), at the organic rice management (O.M.) (A) and conventional rice management (C.M.) (B) from both seasons, 2013/2014 and 2014/2015. Nova Santa Rita, RS, Brazil.

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Fig 8

Rarefaction curves of Platygasteridae parasitoids collected at the organic rice management (O.M.) and conventional rice management (C.M.) from both seasons, 2013/2014 and 2014/2015. Nova Santa Rita, RS, Brazil.

5 ARTIGO 3

**Molecular diversity and phylogeography of *Telenomus podisi* (Hymenoptera:
Platygastridae)***

* Artigo formatado conforme as normas da Zootaxa

Molecular diversity and phylogeography of *Telenomus podisi* (Hymenoptera: Platygasteridae)

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Abstract

Telenomus podisi Ashmead (Hymenoptera: Platygasteridae) is an important species in biological control of agricultural pests in many countries. Despite its importance in agricultural field, some taxonomic questions remain unclear. Thus, more studies are needed to understand the relationships between themselves. The objective of this study was to use population genetic methods in conjunction with two molecular markers: COI and ITS2, to address relationships between *T. podisi* specimens from different biogeographic regions. One hundred and forty-nine specimens identified as *T. podisi* were sampled from 18 localities in seven different countries from 1991 to 2016. Seventy-three COI and ITS2 haplotypes were identified. The haplotypes were the same for both genes. Haplotype 73 (H73) was the most frequent (13%) occurring in 22% of localities. H6 was the second most frequent haplotype (11%) occurring in 28% of localities and H8 represented (8%) also occurring in 28% of localities. Four main clades are revealed in the phylogenetic tree. Haplotype 72 was the most basal to all others haplotypes. In sequence, 3 branches that originate 3 clades (haplotypes H73 to H41) and a monophyletic group formed from H39 to H1 (haplotypes H40 to H1). More than 70% of haplotypes were singletons and 89% unique to a geographic area. The presence of many singletons may indicate a recent origin for them. Haplotype H6 from Arizona, Texas (Bastrop State Park and Cooke County), Ohio and Brazil can be establishing in small populations across geographic area. Haplotype H8 from Texas (Bastrop State Park and Lick Creek Park), Ohio, Cuba and Brazil can be spread out for long time ago with good establishment or/and have more than one population colonizing in the same region. Some haplotypes seem to be evolving through different pathways and

independently of geographic location. Other features may be putting pressure on these haplotypes, such as climatic barriers, shift of host and flora associated. It is important inside biological control context, because differences in each population can lead to different methodologies regard to have an effective biological control.

Key words: Genetic diversity, biological control, biogeography, haplotypes

Introduction

Telenomus podisi Ashmead (Hymenoptera: Platygastridae) is an important species in biological control of agricultural pests in many countries (Corrêa-Ferreira 2002; Ehler 2002). It parasitizes Pentatomidae eggs, many of which are agricultural pests (Hanson & Gauld 2006). In Brazil, it is recorded parasitizing *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae), where parasitism rates up to 75% in rice crops (Maciel *et al.* 2007; Riffel 2007; Idalgo *et al.* 2013). In soybean, the parasitism over *Euschistus heros* (Fabricius), *Podisus connexivus* Bergoth and *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) eggs rates up to 73%, 61% and 50%, respectively (Corrêa-Ferreira & Moscardi 1996). In United States, *T. podisi* showed a higher parasitism over the Pentatomidae *Nezara viridula* (Linnaeus) and *Euschistus servus* (Say) in corn, peanut and cotton farmscapes (Tillman 2011). Also, the important pest brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) has been found parasitized by *T. podisi* (Talamas *et al.* 2015). Despite its importance in agricultural field, some taxonomic questions about this species remain unclear.

Platygastridae belongs to superfamily Platygastroidea with 6011 species described (Hymenoptera online 2016). The subfamily Telenominae includes 905 species, being 652 valid species described in *Telenomus* Haliday genus (Taekul *et al.* 2014). There is some disagreement about the classification of this subfamily (Kozlov 1970; Kozlov & Kononova 1983; Masner 1972; 1976; Masner & Huggert 1979) and the relationships at lower taxonomic levels are also problematic. According to Murphy *et al.* (2007), this subfamily is monophyletic, but some genera like *Telenomus*, showed a nonmonophyly. More recently Taekul *et al.* (2014) also fail to settle the *Telenomus* monophyly and found a nonmonophyly in *podisi* group. Johnson (1984) describe *T. podisi* belongs to Nearctic species with others species within *podisi* group from Neotropical Region, however, in this region diversity is greater and do not show a fixed

diagnostic. So, more studies about this group to try understand taxonomic relationships at a finer level are necessary.

Through the study of genetics population, we can investigate the connection between demographic features and the molecular genetic variants, which influence speciation and taxonomic hierarchy (Sunnucks 2000) and some researches are conducted for this purpose. Miranda *et al.* (2016) identified two population groups of *Partamona rustica* Pedro & Camargo (Hymenoptera: Apidae) in west and east of the São Francisco River in Brazil through four mitochondrial genes and eight microsatellite loci. A paper that examined the impacts of an initial bottleneck and subsequent expansion on genetic structure of introduced wasp *Polistes chinensis antennalis* (Fabricius) (Hymenoptera, Vespidae) in New Zealand from East Asia was conducted by Tsuchida *et al.* (2014). Both researches found differences in genetic structure of studied populations through different geographic distribution.

Studies regarding host location and acceptance have begun to raise questions as to whether *T. podisi* is a single species. A study of *T. podisi* raised from specimens from Brazil and United States found differences between them in responses to host walking traces, cold tolerance, cuticular hydrocarbon composition and cross mating (Borges *et al.* 2003), leading a doubt on the idea that the two populations belong to the same species.

Taxonomy and biological systematics are sciences of discovering, describing, classifying and naming organisms (Tancoigne *et al.* 2011). It is the basic scientific discipline of biology, postulating hypotheses of identity and relationships, on which all other natural sciences dealing with organisms relies (Wägele *et al.* 2011). Taxonomic knowledge is essential whether studying whole organisms, their organs, their specific bio-molecules or biochemical pathways. The study only can be considered robust enough to discuss species-specific traits or attributes, if a species identity is unambiguous (Wägele *et al.* 2011). Identification of a target species of study impacts all subsequent comparisons, predictions, and possible replication of the study (Wägele *et al.* 2011).

Many researchers disagree about the concept of species and the limits of this. De Queiroz (2007) argued that the key to reconciling the alternative species concepts is identifying a common element, which implies a single, more general, concept of species. It refers to equate species with evolving metapopulation lineages separately, or more specifically, with segments of such lineages (De Queiroz 2007). The 'lineage' refers to an ancestor-descendant series (Simpson 1961; Hull 1980) in this case of metapopulations or simply a metapopulation extended

through time (Simpson 1951). So, the evolutionary species concept (ESC), is the primary concept while the others are secondary species criteria (De Queiroz 2007).

Among the genes used as molecular markers to identify species and populations genetics, there are the mitochondrial gene COI (cytochrome oxidase subunit I) and the ITS2 (rDNA internal transcribed spacer 2). These genes have been used to identify many insects species such as *Encarsia* spp. (Hymenoptera: Aphelinidae) (Monti *et al.* 2005), blow flies (Diptera: Calliphoridae) (Khullar *et al.* 2016), *Eumerus* sp. (Diptera: Syrphidae) (Chroni *et al.* 2016); *Lysiphlebus* sp. (Braconidae) (Rahimi *et al.* 2012), *Horismenus* sp. (Eulophidae) (Kenyon *et al.* 2015) and Trichogrammatidae (Almeida & Stouthamer 2015; Venkatesan 2016). Thus, these tools can answer questions about the phylogeny and species delimitation of a group. This is also important because Platygasteridae are biological control agents and the strains may have host specificity. This information can have a major impact on the success of biological control.

The objective of this study is to use population genetic methods in conjunction with two molecular markers: the mitochondrial protein-coding gene COI, and the nuclear protein-coding gene ITS2, to address relationships between *Telenomus podisi* specimens from different biogeography regions.

Materials and Methods

Taxa sampling

One hundred and forty-nine specimens identified as *T. podisi* and one specimen identified as *Trissolcus brochymenae* (Ashmead) (outgroup) based on morphology were sorted from hymenopteran collected by Malaise traps or reared from eggs of stink bugs from 18 localities in seven different countries from 1991 to 2016 (Table 1 and Appendix 1). The label data for all specimens have been georeferenced and recorded in the Hymenoptera On-Line database (Hymenoptera online 2016). Details of these specimens can be accessed entering the identifier in the form (note the space between the acronym and the number). All insects were stored in 95% ethanol at -20 °C until DNA extraction. After nondestructive DNA extraction the voucher specimens were deposited in the C. A. Triplehorn Insect Collection (OSUC).

DNA extraction, amplification, and sequencing

Genomic DNA from whole specimens was extracted using a nondestructive protocol as described in Taekul *et al.* (2014). Following extraction a 597-bp fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) was amplified using the SCEL-F1/HCO-2198 primer pair (Garipey *et al.* 2014), and the entire internal transcribed spacer 2 (ITS2) region was amplified using the ITS2-F/ITS2-R primer pair (Ji *et al.* 2003). Polymerase chain reactions (PCRs) were performed using GoTaq[®] Green Master Mix (Promega) and conducted in a Mastercycler[®] nexus gradient (Eppendorf). Thermocycling conditions were: an initial denaturing step at 95°C for 4 min, followed by 35 cycles of 95°C for 45 s, 54°C for 45 s, 72°C for 45 s and an additional extension at 72°C for 5 min. The PCR products were detected by electrophoresis on a 1.2% agarose gel stained with ethidium bromide and purified using the QIAquick PCR purification kit protocol when necessary. Amplicons were directly sequenced in both directions with forward and reverse primers by Beckman Coulter Genomics (Danvers, MA). Chromatograms were assembled with Sequencher v4.0 (Gene Codes Corporation, Ann Arbor, MI). Sequences for COI and for ITS2 are listed in Appendix 4 and 5, respectively. Sequences will be submitted to GenBank.

Phylogenetic analysis and haplotype network

The sequences of both the COI gene and the ITS2 region were aligned by MAFFT v7 (Kato & Standley 2013) and unique haplotype was determined using DnaSP5 (Librado & Rozas 2009). The genetic differences, including haplotype diversity (h) and nucleotide diversity within populations were estimated by an analysis of molecular variance (AMOVA) using Arlequin 3.5 (Excoffier & Lischer 2010) with 10,000 permutations. Regional haplotype diversity was visualized as pie charts on a map using PhyloGeoViz (Tsai 2010).

Bayesian analyses were performed in MrBayes 3.2 (Ronquist *et al.* 2012) for both genes and the concatenated matrix. jModelTest2 (Darriba *et al.* 2012) was used to test for the best-fit model of evolution for each gene. GTR + I + G was selected for both genes and the concatenated alignment by the corrected Akaike Information Criterion (AICc) (Darriba *et al.* 2012). Two independent Markov chains were run for 5 million generations and convergence was maximized by ensuring that the average standard deviation of split frequencies was below 0.01. Trees were sampled every 1000 generations and the first 25% of each run was discarded as burn-in. The

species *T. brochymenae* was used as outgroup. FigTree version 1.4.3 (Rambaut 2016) and TreeGraph2 (Stöver & Müller 2010) was used to visualize the results.

Results

Spatial distribution of genetic and haplotype diversity

Among the 149 examined individuals representing 18 populations, 73 COI and ITS2 haplotypes (h) were identified (Table 1). The haplotypes were the same for both genes.

Genetic diversity measures include haplotype diversity, i.e. the probability that two randomly chosen haplotypes are different; and nucleotide diversity, i.e. the average number of nucleotide differences per site between two randomly chosen DNA sequences (Nei & Li 1979). For the total sample measures showed high haplotype diversity (h-mean 0.735; range: 0–1.000) and high nucleotide diversity (π -mean 0.053; range: 0–0.199). High haplotype and genetic diversity showed in Ohio (OH), Arkansas (AR), Kansas (KS), Texas Cooke county (TXcc), Texas Lick Creek (TXlc), Cuba (CUBA) and Brazil (BRA RS) samples (h range: 0.667-1.000; π range: 0.064 – 0.199). High haplotype diversity and low genetic diversity showed in Texas Star county (TXsc), Texas Kennedy Ranch (TXkr), Arizona (AZ), Tennessee (TN) and Dominican Republican Armando Bermudez (DRab) (h range: 0.900-1.000; π range: 0.000 – 0.024). Low haplotype and genetic diversity showed in Mexico (MEX), Dominican Republican Morne Trois Pitons (DRmt), Panama (PAN) and Bolivia (BOL) (h:0; π : 0) (Table 1).

Single haplotypes represented 76.7% (56/73) of all haplotypes. Haplotype 73 (H73) was the most frequent, representing 13% (19/149) of the total sample, and being the only haplotype found in Bolivia, Dominican Republic (Morne Trois Pitons), Panama and Mexico. H6 was the second most frequent haplotype representing 11% (16/149), and was found in five localities (AZ, TXbs, TXcc, OH and BRA RS). H8 represented 8% (12/149) and also, was spread among five localities (TXcc, TXlc, OH, Cuba and BRA RS). The remaining frequent haplotypes found in more than one locality was H49, H68, H35, H9 and H19. In total, were found 65 unique haplotypes (89% of total), i.e. haplotypes unique to a geographic area. Geographical distribution of haplotypes diversity is showed in Figure 1.

Phylogenetic and phylogeographic analyses

Three trees were generated by Bayesian Inference analysis with COI genes (Appendix 2), ITS2 region (Appendix 3) and a concatenate tree of both molecular markers (Figure 2). All trees had a high posterior probability values.

Four main polyphyletic clades are revealed in the phylogenetic tree. Haplotype 72 was the most basal to all others haplotypes. In sequence, three branches that originates three clades (haplotypes H73 to H41) and a monophyletic group formed from H39 to H1 (haplotypes H40 to H1). The haplotypes H73 (BOL, PAN, MEX and DRmt) formed the same subclade with H70, H71 and H69 from BRA RS. The next subclade was formed by haplotypes from Ohio, Texas (TXlc and TXkr), Arkansas and Brazil (H65, H67, H68, H66). The next clade was entirely composed of haplotypes detected in the populations placed in Ohio and Tennessee. The third one, is compounded by haplotypes from Brazil (4), Cuba (1) and Texas (1-TXsc). A monophyletic group formed by three subclades have haplotypes from Arkansas (1), Kansas (1), Arizona (4) and Brazil (1) in the first one; Ohio (12), Texas (TXlc-2 and TXcc-3) and Kansas (3) in the second subclade, and in the third one, haplotypes from Ohio (10), Arkansas (1), Texas (TXlc-5, TXcc-3, TXbs-1), Arizona (1), Cuba (1), Drab (1) and Brazil (3) (Figure 2).

Discussion

The large numbers of singleton haplotypes revealed in this study are quite striking. In fact, more than 70% of haplotypes are singletons and 89% are unique to a geographic area. The presence of many singletons may indicate a recent origin for them (Crandal & Templeton 1993) or local adaptation. Contrasting is that the haplotype most frequent H73 is from Mexico, Dominican Republic (Morne Trois Pitons), Panama and Bolivia. All these countries are in Central America, except by Bolivia in the middle South America, and are closer countries compared to USA and South America. These populations had low haplotype and genetic diversity. Population with small haplotype and genetic diversity can be trough recent population bottleneck or founder event by single or a few DNA lineages (Grand & Bowen 1998). A geographical or climatic barrier could prevent many haplotypes from establish in determined local and then increase effective population sizes and a large amount of within-population genetic variation as recorded also for other insects (Ferreira & Ferguson 2010; Tan *et al.* 2016). These results can suggest two pathways for them. First, the population spread out from Central

America through South America or second, the opposite happened with the stopped spread population in Central America by geographic or climatic barriers.

The second most frequent haplotype was H6 (AZ, TXbs, TXcc, OH and BRA RS). In these populations, some have high haplotype diversity and low genetic diversity (TXbs and AZ), that can mean population bottleneck followed by rapid population growth and accumulation of mutations (Grand & Bowen 1998). The genetic diversity of invasive populations is always considered low due to the reduced size of propagules and bottleneck events (Nei *et al.* 1975). Then, these haplotypes can be establishing in small populations across geographic area.

The third most frequent was H8 (TXcc, TXlc, OH, Cuba and BRA RS). All these population have high haplotype and genetic diversity that can mean large stable population with long evolutionary or secondary contact between differentiated lineages (Grand & Bowen, 1998). Then, they can be spread out for long time ago with good establishment or/and have more than one population colonizing in the same region. Multiple invasions/colonization would be an alternative explanation for the high genetic diversity observed (Guidolin *et al.* 2014).

Phylogeography is a powerful tool to infer evolutionary processes at the intraspecific level and among closely related species (Avice 1998, 2009). The use of both cytoplasmic and nuclear markers is preferred over use of a single gene to reveal the phylogeographic patterns (Presa *et al.* 2002; Mardulyn *et al.* 2011; Turmelle *et al.* 2011; Cornetti *et al.* 2014). From the results, we can see through the phylogenetic analysis two different groups within the populations. Some haplotypes seem to be evolving through different pathways and independently of geographic location. Other features may be putting pressure on these haplotypes.

Although it is not clear how mitochondrial DNA selection evolves (Meiklejohn *et al.* 2007), mutation of mitochondrial DNA has been directly linked with phenotypic selection (Ballard & Melvin 2010), resulting in phenotypes expressing diverse fitness traits (Ballard & Melvin 2010; Rand *et al.* 2004). This can occur in spread pest disease, with some haplotype of insect vector being associated with specific haplotype (Guidolin *et al.* 2014). *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) for example, is recognized as a species complex of 34 species that are morphologically indistinguishable (Boykin & De Barro 2014); however, putative species exhibit differences in host range, phylogeographical clustering and potential to transmit viruses (Legg *et al.* 2014; Wainaina *et al.* 2016). Thus, it can occur with parasitoids indistinguishable morphologically.

The shift of host also can be associated with differences on haplotypes. A research with the parasitoid *Pnigalio mediterraneus* Ferriere & Delucchi (Hymenoptera: Eulophidae) showed lower genetic diversity of these species on *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae) than on *Bactrocera oleae* Rossi (Diptera: Tephritidae). That could be explained by a recent shift to *C. ohridella* or by the fact these host feed on a toxic plant, which could serve as a barrier to the adaptation of many haplotypes to this host (Gebiola *et al.* 2014).

Temperature tolerance (climatic barrier) can be observed in different insects species (Gebiola *et al.* 2014; Tan *et al.* 2016). Borges *et al.* (2003) showed differences in *T. podisi* populations from Brazil (Brasilia) and USA (Maryland) in cold tolerance. Although this aspect was not analyzed by the authors, these populations can have differences in haplotype level, as we can see in this work in some haplotypes from Brazil and USA, justifying the thermotolerance between them.

Telenomus podisi is known by parasite Pentatomidae eggs (Johnson 1984; Hanson & Gauld 2006) and others stink bugs in crops. Many individuals in this research were sampled in crops, such as BRA RS (rice crop), CUBA (beans), TN (organic crops), so it can be associated to pests species. On the other hand, samples such as AZ, AR, TX, OH were sampled in natural areas (forest, prairie), so, may have other hosts (Johnson 1984). More researches associating these haplotypes with hosts can indicate genetic differences regard to their hosts.

The genetic variability of the populations, in general, is higher in the origin regions of the species as compared to those where the species was introduced, as exemplified in the works of Garipey *et al.* (2014b) and Xu *et al.* (2014) that describe only a single COI haplotype and two COII haplotypes in specimens collected from the USA and Canada, in contrast with native Asian range, where are recorded 21 COI haplotypes and 18 COII haplotypes of *H. halys* (Hemiptera: Pentatomidae). For biological control of an exotic species, a population of parasitoid with a larger range of haplotypes would be better to try, because of the greater genetic diversity which can lead a good establishment of the introduced parasitoid; or could to improve some better feature from determined area, such as thermotolerance. For example, from present results, to use specimens from Central America or Bolivia with one kind of haplotype is not a great option, unless these species had some specific recognized important fitness trait.

Speciation can be conceptualized in terms of a few general evolutionary processes: mutation, natural selection, migration (or the lack thereof), and genetic drift and the characters affected by those processes, however, are highly diverse (De Queiroz 2007). They may be

genotypic or phenotypic; qualitative or quantitative; selectively advantageous, disadvantageous, or neutral. About the species concept problem, the important point is that changes in these characters lead to the acquisition of a number of different properties by diverging lineages (De Queiroz 2007). In this study, we can see variation within populations that can lead to future speciation. It is important inside biological control context, because can change relation of these populations regarding some host insects (acceptance or not, host shift) (Gebiola *et al.* 2014), as well as local flora and association (Popkin *et al.* 2016), physiological features (Borges *et al.* 2003), insecticides resistance (Vontas *et al.* 2002; Guidolin *et al.* 2014) and transgenic response (Gantz *et al.* 2015; Xia *et al.* 2016).

Differences in each population can lead to different methodologies regarding to biological control. Then, methodological adaptations should be created according to geographical area of work being account intrinsic characteristics of each population to have an effective biological control.

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TABLE 1. Sampling sites with their coordinates and abbreviation, number of individuals (N), haplotype (h) and nucleotide (π) diversity of each populations grouped according to geographical origin of *Telenomus podisi*.

Locality	Abbreviation	Coordinates	N	Number of Haplotypes	h (SD)	π (SD)	Haplotype distribution
USA/ Ohio	OH	39.59936°N 83.02809°W	41	31	0.972 ± 0.016	0.123 ± 0.061	H1(2), H2(1), H3(1), H5(1), H6(1), H8(1), H9(1), H12(1), H14(1), H15(1), H17(1), H18(4), H19(6), H20(1), H21(1), H22(1), H23(1), H24(1), H26(1), H30(1), H40(2), H49(1), H51(1), H52(1), H53(1), H54(1), H55(1), H57(1), H61(1), H68(1)
USA/Arkansas	AR	34.303162°N 93.031013°W	3	3	1.000 ± 0.272	0.199 ± 0.150	H4(1), H35(1), H67(1)
USA/Kansas	KS	39.062476N 96.36329W	4	4	1.000 ± 0.177	0.092 ± 0.0621	H19(1), H27(1), H28(1), H39(1)
USA/Texas, Cooke Co.	TXcc	33.400199N 97.210596W	8	6	0.928 ± 0.084	0.065 ± 0.0368	H6(2), H7(1), H8(2), H19(1), H25(1), 32(1)
USA/Texas, Lick Creek Park	TXlc	30.340198N 96.123917W	12	9	0.935 ± 0.051	0.138 ± 0.072	H8(2), H9(1), H10(1), H13(1), H16(2), H29(1), H33(1), H45(2), H68(1)
USA/Texas, Starr Co.	TXsc	26.371746N 98.445322W	1	1	1.000 ± 0.000	0.000 ± 0.000	H47(1)
USA/Texas, Kenedy Ranch	TXkr	27.133262N 97.474576W	1	1	1.000 ± 0.000	0.000 ± 0.000	H66(1)
USA/Texas, Bastrop State Park	TXbs	30.063665N 97.171284W	1	1	1.000 ± 0.000	0.000 ± 0.000	H6(1)
USA/Arizona, Appleton Whittell	AZ	31.361156N 110.302686W	5	4	0.900 ± 0.161	0.006 ± 0.005	H35(2), H36(1), H37(1), H38(1)
USA/Arizona, Coronado National Forest	AZ	31.441505N 110.453574W	1	1	1.000 ± 0.000	0.000 ± 0.000	H6(1)
USA/Tennessee, Knox Co.	TN	35.591487N 83.583112W	11	9	0.964 ± 0.051	0.025 ± 0.014	H48(1), H49(1), H50(1), H56(1), H58(1), H59(2), H60(1), H62(2), H63(1)

...Continued on the next page

TABLE 1. (Continued)

Locality	Abbreviation	Coordinates	N	Number of Haplotypes	h (SD)	π (SD)	Haplotype distribution
Mexico/Oaxaca	MEX	16.042812N 96.28099W	4	1	0.000 \pm 0.000	0.000 \pm 0.000	H73(4)
Cuba/Manicaragua	CUBA	22.084796N 79.584086W	3	2	0.667 \pm 0.314	0.127 \pm 0.096	H8(2), H44(1)
Dominican Republic/ Armando Bermúdez National Park	DRab	19.103281N 71.030015W	1	1	1.000 \pm 0.000	0.000 \pm 0.000	H11(1)
Dominican Republic/Morne Trois Pitons NP	DRmt	15.20953N 61.20556W	3	1	0.000 \pm 0.000	0.000 \pm 0.000	H73(3)
Panama/Veraguas	PAN	8.3031N 81.0710W	3	1	0.000 \pm 0.000	0.000 \pm 0.000	H73(3)
Bolivia/Santa cruz	BOL	17.50124S 64.23454W	9	1	0.000 \pm 0.000	0.000 \pm 0.000	H73(9)
Brazil/Rio Grande do Sul	BRA RS	29.471662S 51.210091W	38	14	0.859 \pm 0.035	0.180 \pm 0.089	H6(11), H8(5), H9(1), H34(1), H41(8), H42(1), H43(1), H46(1), H64(1), H65(1), H69(4), H70(1), H71(1), H72(1)
Average					0.735 \pm 0.645	0.053 \pm 0.033	

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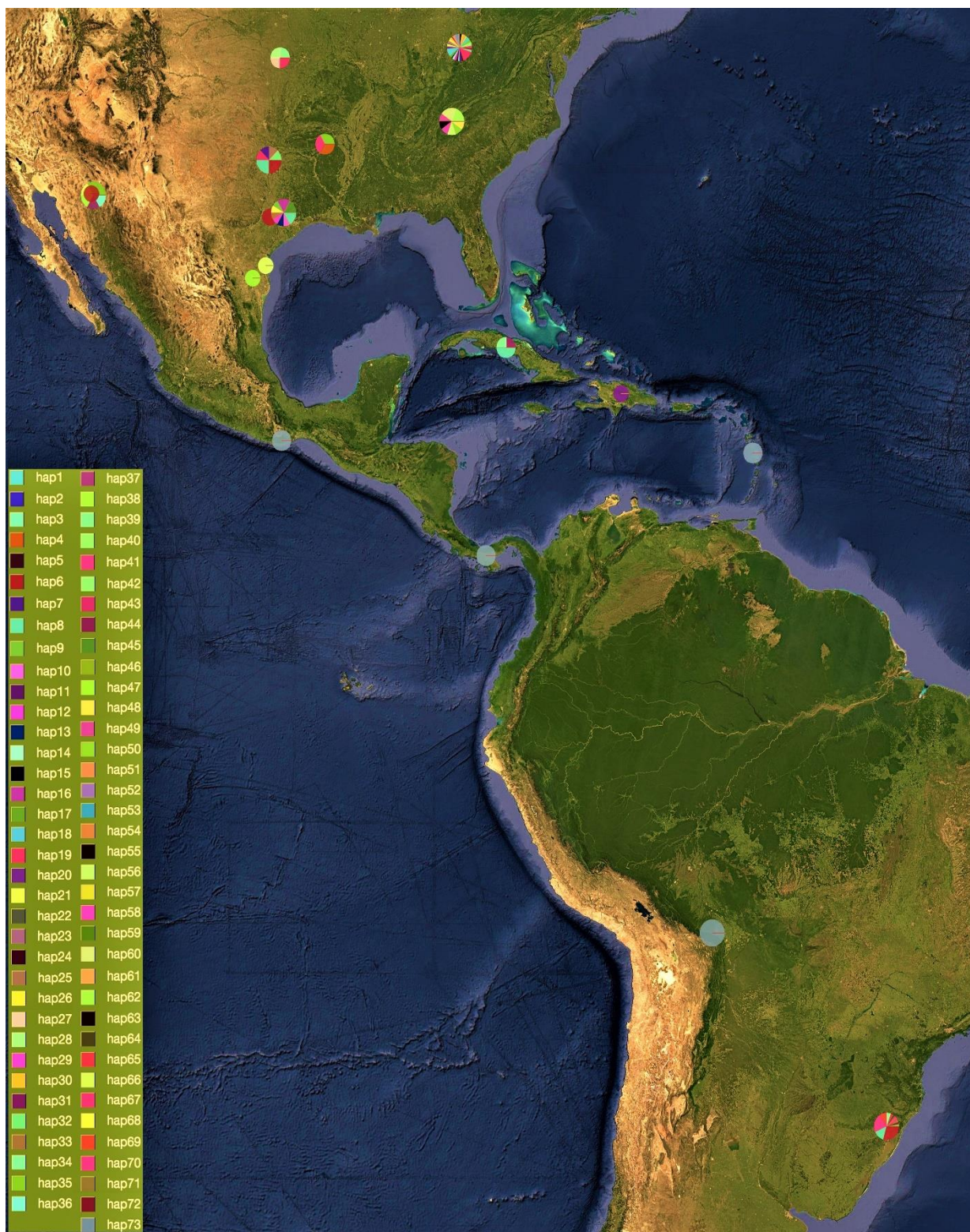


FIGURE 1. Geographic distribution of the 73 haplotypes among the 18 sampled localities. Each pie represents a locality.

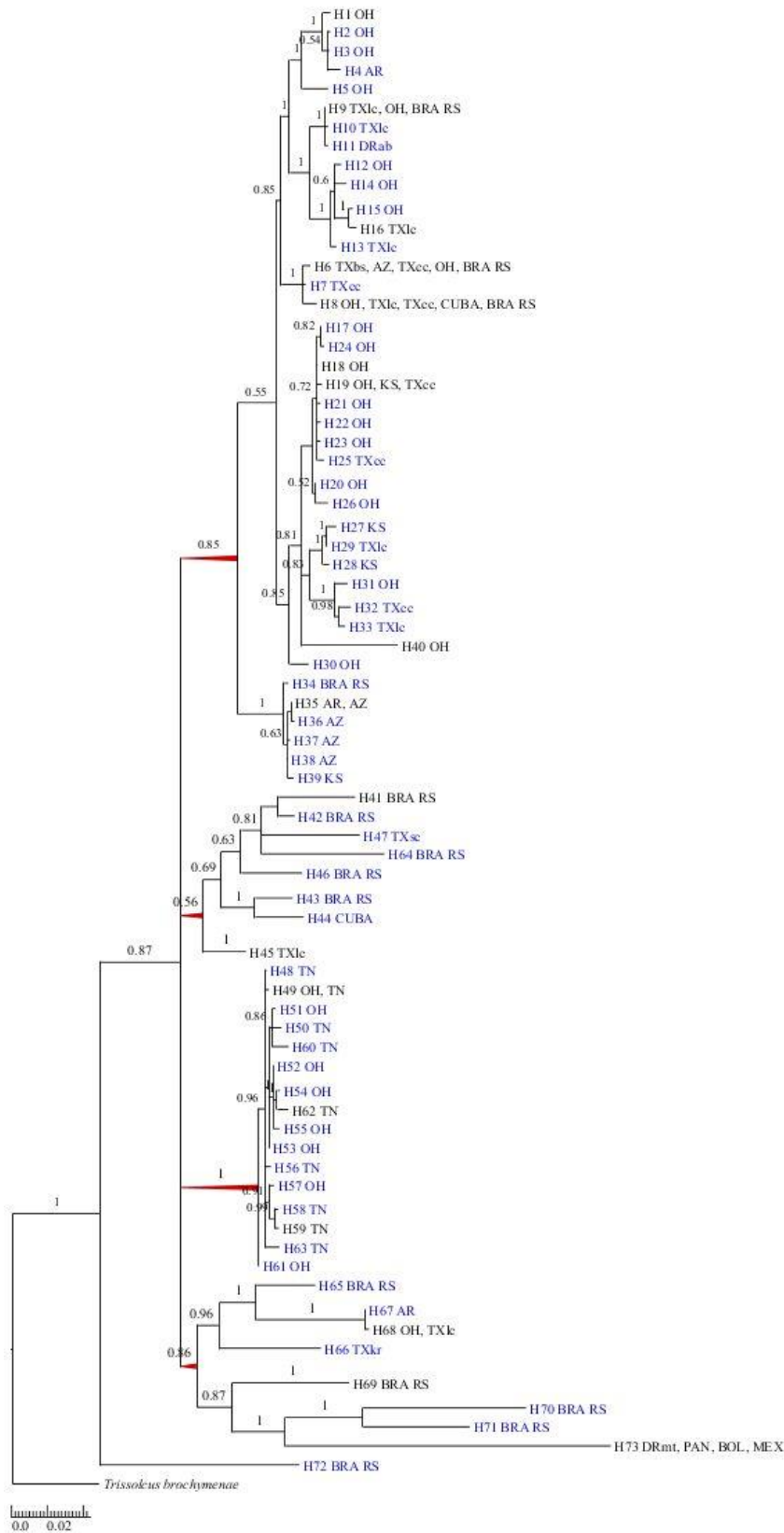


FIGURE 2. Bayesian consensus concatenate tree of the COI gene and ITS2 haplotypes of *Telenomus podisi* with posterior probability values showed over the branches. *Branches of the four main clades are in red color and singletons haplotypes are highlighted in blue color.

6 CONSIDERAÇÕES FINAIS

As diferenças apontadas neste trabalho a respeito do gradiente de distância da vegetação nativa em relação à lavoura do arroz indicam que fragmentos de vegetação nativa são importantes para a diversidade e abundância de himenópteros parasitoides, principalmente em áreas do manejo convencional, onde a vegetação nas taipas não está presente. Nas áreas com manejo orgânico, nas quais as taipas possuem vegetação espontânea, esta influência não é claramente percebida, possivelmente porque estas servem de refúgio, abrigo e corredor ecológico para os inimigos naturais.

Os estágios fenológicos de desenvolvimento do arroz podem influenciar a composição de famílias de parasitoides e, dentro da família Platygasteridae (Scelioninae, Teleasinae e Telenominae) especificamente, com diferentes morfoespécies e em densidades discrepantes nos dois períodos da cultura. Esse aspecto deve estar relacionado aos diferentes hospedeiros, muitas vezes insetos fitófagos, presentes em cada fase da cultura. Investigações referentes a isso podem trazer importantes informações a respeito da dinâmica e das relações destas populações no sistema orizícola.

Os inseticidas químicos podem alterar a riqueza e abundância de parasitoides nas áreas de arroz, o que foi constatado em razão da mudança do manejo realizado na área do convencional pelo produtor que, na primeira safra utilizou inseticidas considerados seletivos aos

inimigos naturais e, na segunda aplicou produtos não seletivos (neurotóxicos), decrescendo a abundância de parasitoides no local.

Os registros deste trabalho destacam a importância da família Platygasteridae para o controle biológico de pragas em áreas de arroz, indicando que um controle biológico natural pode estar ocorrendo no local, principalmente na área do manejo orgânico.

O terceiro capítulo assinala a variação entre as populações de *T. podisi* e esboça aspectos que conduzem a uma futura especiação. Isto é importante dentro do contexto do controle biológico, pois essas variações podem levar a mudanças nas relações dos parasitoides com determinados insetos hospedeiros, planta hospedeira, características fisiológicas e resistência a inseticidas. Assim, adaptações metodológicas deveriam ser desenvolvidas de acordo com a área geográfica de trabalho considerando características intrínsecas de cada população específica para se ter um efetivo controle biológico.

7 APÊNDICES

APÊNDICE 1. List of specimens included in this study.

Taxa	Lab Code	Voucher no.	COI	ITS2
<i>Telenomus podisi</i>	G0002	OSUC 555922	Y	Y
<i>Telenomus podisi</i>	K0025	OSUC 372813	Y	Y
<i>Telenomus podisi</i>	K0020	OSUC 372810	Y	Y
<i>Telenomus podisi</i>	K0044	OSUC 557737	Y	Y
<i>Telenomus podisi</i>	K0034	OSUC 372818	Y	Y
<i>Telenomus podisi</i>	K0032	OSUC 557729	Y	Y
<i>Telenomus podisi</i>	K0040	OSUC 557733	Y	Y
<i>Telenomus podisi</i>	K0027	OSUC 557726	Y	Y
<i>Telenomus podisi</i>	K0019	OSUC 557723	Y	Y
<i>Telenomus podisi</i>	K0024	OSUC 372812	Y	Y
<i>Telenomus podisi</i>	K0042	OSUC 557735	Y	Y
<i>Telenomus podisi</i>	G0003	OSUC 555923	Y	Y
<i>Telenomus podisi</i>	K0001	OSUC 372802	Y	Y
<i>Telenomus podisi</i>	K0033	OSUC 372817	Y	Y
<i>Telenomus podisi</i>	K0037	OSUC 372819	Y	Y
<i>Telenomus podisi</i>	K0009	OSUC 557716	Y	Y
<i>Telenomus podisi</i>	K0038	OSUC 557732	Y	Y
<i>Telenomus podisi</i>	K0005	OSUC 557715	Y	Y
<i>Telenomus podisi</i>	K0016	OSUC 557722	Y	Y
<i>Telenomus podisi</i>	K0046	OSUC 557739	Y	Y
<i>Telenomus podisi</i>	K0047	OSUC 372821	Y	Y
<i>Telenomus podisi</i>	K0014	OSUC 557720	Y	Y
<i>Telenomus podisi</i>	K0015	OSUC 557721	Y	Y
<i>Telenomus podisi</i>	K0041	OSUC 557734	Y	Y
<i>Telenomus podisi</i>	K0045	OSUC 557738	Y	Y
<i>Telenomus podisi</i>	K0051	OSUC 557743	Y	Y
<i>Telenomus podisi</i>	K0013	OSUC 372807	Y	Y
<i>Telenomus podisi</i>	K0039	OSUC 372820	Y	Y
<i>Telenomus podisi</i>	K0018	OSUC 372809	Y	Y
<i>Telenomus podisi</i>	K0002	OSUC 557713	Y	Y
<i>Telenomus podisi</i>	K0017	OSUC 372808	Y	Y

continuação APÊNDICE 1. List of specimens included in this study.

Taxa	Lab Code	Voucher no.	COI	ITS2
<i>Telenomus podisi</i>	K0008	OSUC 372806	Y	Y
<i>Telenomus podisi</i>	K0011	OSUC 557718	Y	Y
<i>Telenomus podisi</i>	K0048	OSUC 557740	Y	Y
<i>Telenomus podisi</i>	K0049	OSUC 557741	Y	Y
<i>Telenomus podisi</i>	K0050	OSUC 557742	Y	Y
<i>Telenomus podisi</i>	K0004	OSUC 372803	Y	Y
<i>Telenomus podisi</i>	K0023	OSUC 557725	Y	Y
<i>Telenomus podisi</i>	K0003	OSUC 557714	Y	Y
<i>Telenomus podisi</i>	K0035	OSUC 557730	Y	Y
<i>Telenomus podisi</i>	K0095	OSUC 372832	Y	Y
<i>Telenomus podisi</i>	K0053	OSUC 557744	Y	Y
<i>Telenomus podisi</i>	K0054	OSUC 557745	Y	Y
<i>Telenomus podisi</i>	K0052	OSUC 372823	Y	Y
<i>Telenomus podisi</i>	K0068	OSUC 557756	Y	Y
<i>Telenomus podisi</i>	K0066	OSUC 557754	Y	Y
<i>Telenomus podisi</i>	K0067	OSUC 557755	Y	Y
<i>Telenomus podisi</i>	K0065	OSUC 557753	Y	Y
<i>Telenomus podisi</i>	K0071	OSUC 557758	Y	Y
<i>Telenomus podisi</i>	K0077	OSUC 557764	Y	Y
<i>Telenomus podisi</i>	K0078	OSUC 557765	Y	Y
<i>Telenomus podisi</i>	K0070	OSUC 557757	Y	Y
<i>Telenomus podisi</i>	K0073	OSUC 557760	Y	Y
<i>Telenomus podisi</i>	K0069	OSUC 372826	Y	Y
<i>Telenomus podisi</i>	K0075	OSUC 557762	Y	Y
<i>Telenomus podisi</i>	K0072	OSUC 557759	Y	Y
<i>Telenomus podisi</i>	K0061	OSUC 557750	Y	Y
<i>Telenomus podisi</i>	K0085	OSUC 557770	Y	Y
<i>Telenomus podisi</i>	K0063	OSUC 557752	Y	Y
<i>Telenomus podisi</i>	K0059	OSUC 557748	Y	Y
<i>Telenomus podisi</i>	K0060	OSUC 557749	Y	Y
<i>Telenomus podisi</i>	K0082	OSUC 557767	Y	Y
<i>Telenomus podisi</i>	K0087	OSUC 557772	Y	Y
<i>Telenomus podisi</i>	K0084	OSUC 557769	Y	Y
<i>Telenomus podisi</i>	K0081	OSUC 372828	Y	Y
<i>Telenomus podisi</i>	K0086	OSUC 557771	Y	Y
<i>Telenomus podisi</i>	K0094	OSUC 557776	Y	Y
<i>Telenomus podisi</i>	K0092	OSUC 372831	Y	Y
<i>Telenomus podisi</i>	K0090	OSUC 557774	Y	Y
<i>Telenomus podisi</i>	K0091	OSUC 372830	Y	Y
<i>Telenomus podisi</i>	K0096	OSUC 372833	Y	Y
<i>Telenomus podisi</i>	K0104	OSUC 372836	Y	Y
<i>Telenomus podisi</i>	K0108	OSUC 557785	Y	Y

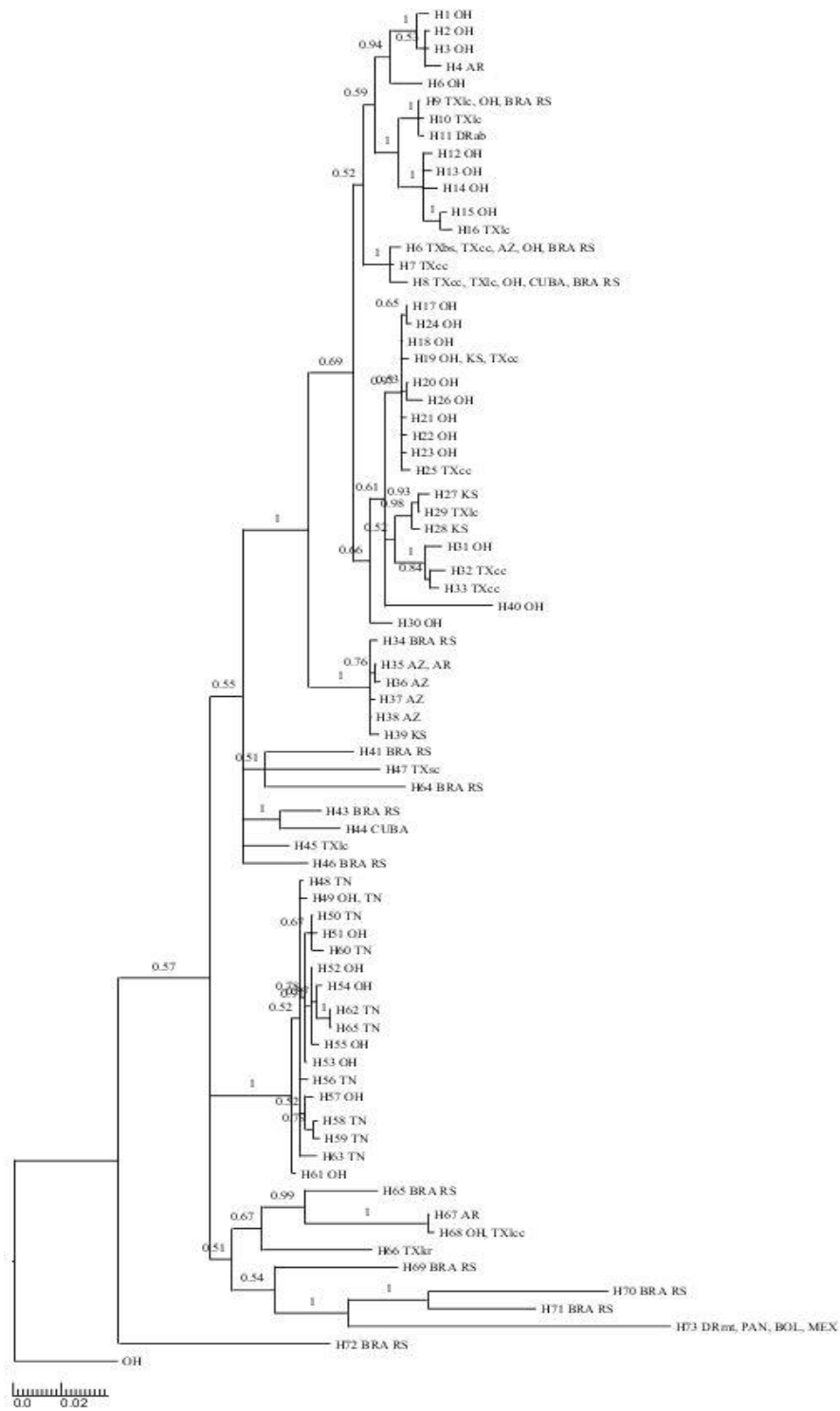
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Taxa	Lab Code	Voucher no.	COI	ITS2
<i>Telenomus podisi</i>	K0105	OSUC 557782	Y	Y
<i>Telenomus podisi</i>	K0106	OSUC 557783	Y	Y
<i>Telenomus podisi</i>	K0107	OSUC 557784	Y	Y
<i>Telenomus podisi</i>	K0080	OSUC 557766	Y	Y
<i>Telenomus podisi</i>	G0095	OSUC 553316	Y	Y
<i>Telenomus podisi</i>	G0108	OSUC 553324	Y	Y
<i>Telenomus podisi</i>	G0106	OSUC 553322	Y	Y
<i>Telenomus podisi</i>	G0101	BMSB 1155	Y	Y
<i>Telenomus podisi</i>	G0097	BMSB 1146	Y	Y
<i>Telenomus podisi</i>	G0098	OSUC 553318	Y	Y
<i>Telenomus podisi</i>	G0109	OSUC 553325	Y	Y
<i>Telenomus podisi</i>	G0104	BMSB 1157	Y	Y
<i>Telenomus podisi</i>	G0103	OSUC 553320	Y	Y
<i>Telenomus podisi</i>	G0105	OSUC 553321	Y	Y
<i>Telenomus podisi</i>	G0100	OSUC 553319	Y	Y
<i>Telenomus podisi</i>	G0005	OSUC 553194	Y	Y
<i>Telenomus podisi</i>	G0007	OSUC 553196	Y	Y
<i>Telenomus podisi</i>	G0008	OSUC 553197	Y	Y
<i>Telenomus podisi</i>	G0009	OSUC 553198	Y	Y
<i>Telenomus podisi</i>	G0014	OSUC 553203	Y	Y
<i>Telenomus podisi</i>	G0017	OSUC 553206	Y	Y
<i>Telenomus podisi</i>	G0018	OSUC 553207	Y	Y
<i>Telenomus podisi</i>	G0023	OSUC 553212	Y	Y
<i>Telenomus podisi</i>	G0024	OSUC 553213	Y	Y
<i>Telenomus podisi</i>	G0027	OSUC 553216	Y	Y
<i>Telenomus podisi</i>	G0072	OSUC 553293	Y	Y
<i>Telenomus podisi</i>	G0042	OSUC 553263	Y	Y
<i>Telenomus podisi</i>	G0044	OSUC 553265	Y	Y
<i>Telenomus podisi</i>	G0045	OSUC 553266	Y	Y
<i>Telenomus podisi</i>	G0090	OSUC 553311	Y	Y
<i>Telenomus podisi</i>	G0094	OSUC 553315	Y	Y
<i>Telenomus podisi</i>	G0020	OSUC 553209	Y	Y
<i>Telenomus podisi</i>	G0030	OSUC 553219	Y	Y
<i>Telenomus podisi</i>	G0016	OSUC 553205	Y	Y
<i>Telenomus podisi</i>	G0032	OSUC 553221	Y	Y
<i>Telenomus podisi</i>	G0070	OSUC 553291	Y	Y
<i>Telenomus podisi</i>	G0073	OSUC 553294	Y	Y
<i>Telenomus podisi</i>	G0080	OSUC 553301	Y	Y
<i>Telenomus podisi</i>	G0081	OSUC 553302	Y	Y
<i>Telenomus podisi</i>	G0082	OSUC 553303	Y	Y
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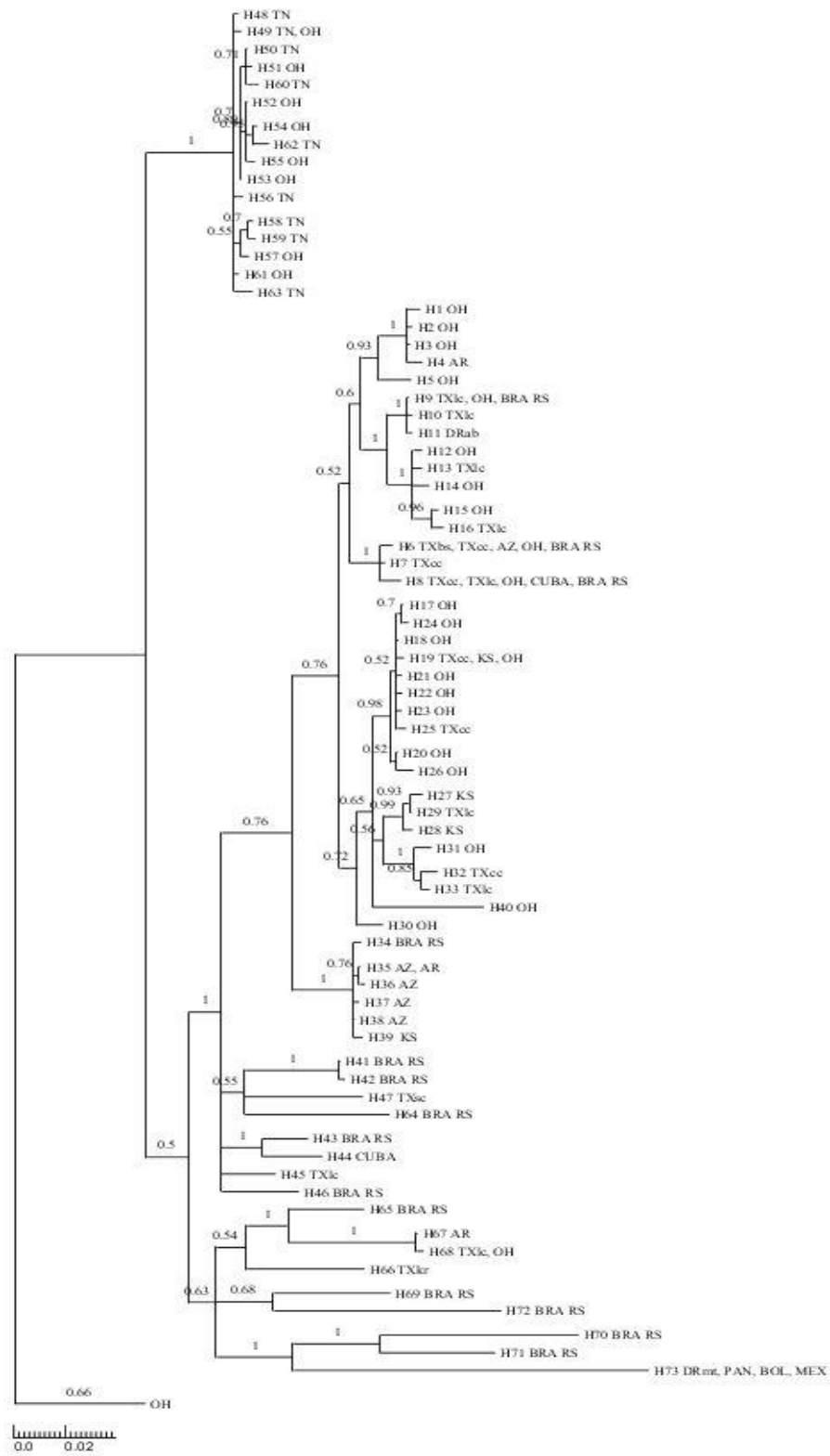
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Taxa	Lab Code	Voucher no.	COI	ITS2
<i>Telenomus podisi</i>	G0063	OSUC 553284	Y	Y
<i>Telenomus podisi</i>	G0065	OSUC 553286	Y	Y
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<i>Telenomus podisi</i>	G0086	OSUC 553307	Y	Y
<i>Telenomus podisi</i>	G0011	OSUC 553200	Y	Y
<i>Telenomus podisi</i>	G0019	OSUC 553208	Y	Y
<i>Telenomus podisi</i>	G0052	OSUC 553273	Y	Y
<i>Telenomus podisi</i>	G0054	OSUC 553275	Y	Y
<i>Telenomus podisi</i>	G0012	OSUC 553201	Y	Y
<i>Telenomus podisi</i>	G0026	OSUC 553215	Y	Y
<i>Telenomus podisi</i>	G0028	OSUC 553217	Y	Y
<i>Telenomus podisi</i>	G0112	OSUC 553326	Y	Y
<i>Telenomus podisi</i>	G0113	OSUC 553327	Y	Y
<i>Telenomus podisi</i>	G0114	OSUC 553328	Y	Y
<i>Telenomus podisi</i>	G0156	OSUC 599344	Y	Y
<i>Telenomus podisi</i>	G0157	OSUC 599345	Y	Y
<i>Telenomus podisi</i>	G0158	OSUC 599346	Y	Y
<i>Telenomus podisi</i>	K0100	OSUC 372835	Y	Y
<i>Telenomus podisi</i>	G0159	OSUC 599347	Y	Y
<i>Telenomus podisi</i>	G0161	OSUC 599349	Y	Y
<i>Telenomus podisi</i>	G0162	OSUC 599350	Y	Y
<i>Telenomus podisi</i>	G0171	OSUC 599359	Y	Y
<i>Telenomus podisi</i>	G0172	OSUC 599360	Y	Y
<i>Telenomus podisi</i>	G0173	OSUC 599361	Y	Y
<i>Telenomus podisi</i>	G0174	OSUC 599362	Y	Y
<i>Telenomus podisi</i>	G0175	OSUC 599363	Y	Y
<i>Telenomus podisi</i>	G0176	OSUC 599364	Y	Y
<i>Telenomus podisi</i>	G0178	OSUC 599366	Y	Y
<i>Telenomus podisi</i>	G0179	OSUC 599367	Y	Y
<i>Telenomus podisi</i>	G0180	OSUC 599368	Y	Y
<i>Telenomus podisi</i>	G0181	OSUC 599369	Y	Y
<i>Telenomus podisi</i>	G0183	OSUC 599371	Y	Y
<i>Telenomus podisi</i>	G0184	OSUC 599372	Y	Y
<i>Telenomus podisi</i>	G0185	OSUC 599373	Y	Y
<i>Trissolcus brochymenae</i>	K0055	OSUC 372822	Y	Y

APÊNDICE 2. Bayesian consensus tree of the COI haplotypes with posterior probability values showed over the branches.



APÊNDICE 3. Bayesian consensus tree of the ITS2 haplotypes with posterior probability values showed over the branches.



APÊNDICE 4. Individuals number in each parasitoid family, sample spot and rice management (Organic = Org; Conventional = Conv) in both crop seasons.

Samples spots	1° Crop Season										2° Crop Season									
	0m (5)		100m (4)		200m (3)		300m (2)		400m (1)		0m (5)		200m (4)		400m (3)		600m (2)		800m (1)	
Families	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv
Platygastridae	31	10	51	49	47	67	77	27	62	22	25	21	55	20	14	10	37	50	48	14
Mymaridae	26	3	41	57	25	42	62	30	31	13	19	19	72	18	39	20	48	18	55	5
Encyrtidae	10	7	29	58	19	48	33	63	30	33	11	7	15	4	6	7	12	9	18	6
Trichogrammatidae	9	2	27	42	30	35	31	29	36	26	7	2	13	14	7	7	15	21	10	6
Eulophidae	5	2	67	11	21	6	19	7	18	1	4	17	71	2	66	3	52	2	77	3
Ceraphronidae	5	20	4	11	11	13	3	14	4	10	8	5	5	2	3	0	1	5	4	1
Ichneumonidae	28	2	19	4	11	6	9	5	5	0	45	5	16	1	10	1	3	4	16	1
Braconidae	5	3	15	9	7	6	10	16	12	2	10	4	11	1	4	10	6	6	21	6
Figitidae	1	0	9	5	5	5	11	2	4	1	4	3	10	1	6	3	5	2	4	2
Diapriidae	0	3	4	2	5	3	3	0	2	1	2	2	4	0	1	1	1	0	1	1
Bethylidae	2	2	4	0	3	1	4	0	6	0	8	4	0	2	2	0	1	0	1	0
Chalcididae	0	0	4	7	2	0	4	0	2	0	0	1	5	0	1	0	2	1	6	2
Eupelmidae	3	0	1	2	2	1	4	1	0	0	6	2	1	0	0	0	0	0	5	0
Aphelinidae	0	0	6	0	0	2	3	0	1	0	0	0	1	0	2	0	0	0	0	1
Pteromalidae	0	3	1	2	1	0	0	0	1	2	1	0	5	0	2	0	1	1	4	0
Eurytomidae	3	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Chrysididae	0	0	0	0	4	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0
Evanidae	4	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0
Torymidae	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryinidae	0	0	2	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0
Megaspilidae	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Signiphoridae	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	1	0
Proctrotupidae	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Total	135	57	285	261	194	236	273	195	217	111	126	74	233	45	150	52	150	69	226	34

APÊNDICE 5. Individuals number in each parasitoid family, samples and rice management in the first crop season (2013/2014).

Families	CONVENTIONAL									ORGANIC							
	Vegetative					Reproductive				Vegetative				Reproductive			
	12/12/13	27/12/13	9/1/14	22/1/14	5/2/14	19/2/14	7/3/14	28/3/14	16/4/14	27/12/13	9/1/14	22/1/14	5/2/14	19/2/14	11/3/14	28/3/14	25/4/14
Platygastridae	14	13	30	12	12	11	30	26	27	17	49	18	30	17	64	60	13
Braconidae	6	3	6	1	0	1	2	2	15	3	10	4	3	1	14	10	4
Eulophidae	1	1	7	1	2	4	4	3	4	10	10	9	12	8	23	38	20
Trichogrammatidae	9	16	17	6	8	15	15	38	10	8	11	18	28	14	19	31	4
Aphelinidae	0	0	0	1	0	0	0	1	0	1	2	0	2	0	0	3	2
Encyrtidae	11	5	5	3	3	1	92	54	35	14	11	10	13	6	42	18	7
Mymaridae	46	6	26	6	18	7	3	28	5	14	21	21	38	12	38	31	10
Ichneumonidae	2	1	9	0	0	1	0	0	4	4	20	10	8	3	5	14	8
Ceraphronidae	2	2	5	4	5	18	21	5	6	0	2	4	5	5	4	5	2
Diapriidae	2	0	3	2	1	1	0	0	0	2	1	1	2	3	2	2	1
Chalcididae	1	1	2	0	1	0	2	0	0	1	0	0	5	2	0	3	1
Megaspilidae	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Eupelmidae	0	0	1	0	1	0	1	1	0	1	4	1	2	1	0	1	0
Bethylidae	0	0	1	0	1	0	0	1	0	2	6	3	3	2	1	2	0
Figitidae	6	2	4	1	0	0	0	0	0	2	11	1	4	1	6	5	0
Torymidae	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0
Dryinidae	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Chrysididae	0	0	0	0	0	0	0	0	0	0	2	0	1	1	1	0	0
Pteromalidae	2	1	1	0	1	1	0	0	1	1	1	1	0	0	0	0	0
Signiphoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Evaniidae	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	2	0
Eurytomidae	1	0	0	0	0	0	0	0	0	0	0	3	0	0	2	0	0
Total	103	51	119	37	53	60	171	159	107	84	163	104	158	76	221	226	72

APÊNDICE 6. Individuals number in each parasitoid family, samples and rice management in the second crop season (2014/2015).

Families	CONVENTIONAL										ORGANIC							
	Vegetative					Reproductive					Vegetative				Reproductive			
	17/12/14	24/12/14	7/1/15	22/1/15	5/2/15	15/2/15	5/3/15	13/3/15	20/3/15	28/11/14	17/12/14	7/1/15	22/1/15	5/2/15	15/2/15	4/3/15	13/3/15	20/3/15
Platygastridae	10	4	22	24	1	6	10	22	16	18	11	30	21	10	40	9	36	4
Braconidae	1	2	3	14	3	4	0	0	0	2	7	7	5	2	3	6	16	4
Eulophidae	5	0	7	5	2	4	1	3	0	14	16	28	14	9	47	40	83	19
Trichogrammatidae	5	0	11	5	3	21	2	0	3	4	1	9	5	3	11	5	13	1
Aphelinidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Encyrtidae	1	0	12	2	4	1	9	4	0	5	3	4	6	2	14	6	21	1
Mymaridae	6	3	14	14	10	9	6	12	6	24	15	8	25	9	43	30	70	9
Ichneumonidae	2	2	1	1	0	2	1	1	2	10	13	2	5	4	4	16	24	12
Ceraphronidae	1	0	6	0	0	1	3	2	0	0	6	2	4	0	2	2	4	1
Diapriidae	2	0	1	0	0	0	1	0	0	1	1	1	1	0	0	5	0	0
Chalcididae	1	0	2	0	0	0	1	0	0	0	1	0	0	1	4	0	4	4
Eupelmidae	0	0	0	1	1	0	0	0	0	1	0	1	0	1	0	2	4	3
Bethylidae	1	0	2	1	0	1	0	0	1	6	1	0	0	0	1	1	3	0
Figitidae	0	0	6	2	1	1	0	0	1	3	5	1	2	3	6	1	6	2
Dryinidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0
Chrysididae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0
Pteromalidae	0	0	0	0	0	0	0	0	1	1	0	3	2	1	4	0	1	1
Signiphoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0
Evaniidae	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0
Proctrotupidae	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0
Total	36	11	87	69	25	52	35	44	30	90	81	96	92	47	183	124	290	61

continuação APÊNDICE 7. Individuals number in each platygastroid morphospecies, sample spot and rice management (Organic = Org; Conventional = Conv) in both crop seasons.

Samples spots	1° Crop Season										2° Crop Season									
	0 (5)		100 (4)		200 (3)		300 (2)		400 (1)		0 (5)		200 (4)		400 (3)		600 (2)		800 (1)	
	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv	Org	Conv
Morphospecies																				
<i>Macroteleia</i> sp.1	0	0	4	3	0	1	3	0	4	2	0	0	1	0	0	0	1	0	0	1
<i>Opisthacantha</i> sp.1	1	0	0	0	2	1	1	2	2	0	2	0	2	0	0	0	1	1	0	0
<i>Paridris</i> sp.1	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	0	0	0
<i>Phanuromyia</i> sp.1	6	0	0	0	3	1	0	0	0	0	0	0	2	2	2	0	0	0	3	0
<i>Probaryconus</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
<i>Probaryconus</i> sp.2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Scelio</i> sp.1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trimorus</i> sp.1	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0
<i>Trimorus</i> sp.2	3	0	4	3	0	1	3	1	2	2	1	1	0	0	0	1	1	0	0	0
<i>Trissolcus</i> sp.1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0
<i>Trissolcus</i> sp.2	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trissolcus</i> sp.3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Triteleia</i> sp.1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Telenomus</i> sp.1	0	1	2	2	3	6	4	1	4	1	1	2	3	6	0	1	5	7	3	1
<i>Telenomus</i> sp.2	1	0	2	0	0	3	7	1	2	0	0	0	2	0	1	1	3	0	8	0
<i>Telenomus</i> sp.3	4	0	13	3	11	11	14	6	15	6	2	1	2	2	1	0	6	3	1	0
<i>Telenomus</i> sp.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0
<i>Telenomus</i> sp.5	3	1	0	2	3	5	2	0	3	1	0	1	0	0	0	0	2	0	0	0
<i>Telenomus podisi</i>	8	0	14	30	19	24	30	5	13	7	1	2	22	2	3	4	3	32	7	6
<i>Telenomus</i> sp.7	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Telenomus</i> sp.8	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Xenomerus</i> sp.1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	32	8	46	50	47	66	81	25	62	23	16	12	49	16	13	10	36	45	37	10

continuação APÊNDICE 8. Individuals number in each platygastroids genera, samples and rice management in the first crop season (2013/2014).

Morphospecies	Vegetative					Reproductive				Vegetative					Reproductive		
	12/12/13	27/12/13	9/1/14	22/1/14	5/2/14	19/2/14	7/3/14	28/3/14	16/4/14	27/12/13	9/1/14	22/1/14	5/2/14	19/2/14	11/3/14	28/3/14	25/4/14
<i>Macroteleia</i> sp.1	0	0	0	2	0	0	2	1	1	2	0	1	0	0	1	7	0
<i>Opisthacantha</i> sp.1	0	0	1	0	0	1	0	0	1	1	0	1	0	1	2	1	0
<i>Paridris</i> sp.1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
<i>Phanuromyia</i> sp.1	0	0	0	0	0	0	0	0	1	0	2	2	2	1	0	2	0
<i>Probaryconus</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Probaryconus</i> sp.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scelio</i> sp.1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0
<i>Trimorus</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Trimorus</i> sp.2	0	0	1	0	3	2	1	0	0	3	2	0	6	0	0	0	1
<i>Trissolcus</i> sp.1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trissolcus</i> sp.2	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Trissolcus</i> sp.3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Triteleia</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
<i>Telenomus</i> sp.1	1	0	2	1	1	2	0	4	0	0	5	0	3	0	0	4	1
<i>Telenomus</i> sp.2	0	0	2	0	1	0	0	0	1	2	5	1	1	0	2	1	0
<i>Telenomus</i> sp.3	1	2	1	1	5	1	6	3	6	0	3	3	4	2	25	17	3
<i>Telenomus</i> sp.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Telenomus</i> sp.5	1	0	8	0	0	0	0	0	0	0	3	0	2	0	4	2	0
<i>Telenomus podisi</i>	1	6	9	3	2	1	18	11	15	3	17	1	3	7	25	22	6
<i>Telenomus</i> sp.7	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Telenomus</i> sp.8	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
<i>Xenomerus</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
TOTAL	12	12	30	11	13	10	29	29	26	16	44	16	29	16	67	68	12

APÊNDICE 9. Individuals number in each platygastroids genera, samples and rice management in the second crop season (2014/2015).

Morphospecies	CONVENTIONAL										ORGANIC							
	Vegetative					Reproductive					Vegetative				Reproductive			
	17/12/14	24/12/14	7/1/15	22/1/15	5/2/15	11/2/15	5/3/15	13/3/15	20/3/15	28/11/14	17/12/14	7/1/15	22/1/15	5/2/15	11/2/15	4/3/15	13/3/15	20/3/15
<i>Anteris</i> sp.1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baeus</i> sp.1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0
<i>Baeus</i> sp.2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Baeus</i> sp.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Baeus</i> sp.4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0
<i>Baeus</i> sp.5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baryconus</i> sp.1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0
<i>Calliscelio</i> sp.1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Calotelea</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Cremastobaeus</i> sp.1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0
<i>Cremastobaeus</i> sp.2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	1	1
<i>Duta</i> sp.1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0
<i>Duta</i> sp.2	0	0	1	0	0	0	0	0	0	0	0	1	1	1	2	0	0	0
<i>Eumicrosoma</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
<i>Gryon</i> sp.1	2	0	0	0	0	0	0	0	0	0	0	1	2	0	2	1	1	0
<i>Gryon</i> sp.2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
<i>Idris</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Idris</i> sp.2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
<i>Idris</i> sp.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
<i>Idris</i> sp.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Idris</i> sp.5	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Idris</i> sp.6	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
<i>Idris</i> sp.7	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
<i>Idris</i> sp.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Idris</i> sp.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Idris</i> sp.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Idris</i> sp.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macroteleia</i> sp.1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
<i>Opisthacantha</i> sp.1	1	0	0	0	0	0	0	0	0	1	0	3	1	0	0	0	0	0

continuação APÊNDICE 9. Individuals number in each platygastroids genera, samples and rice management in the second crop season (2014/2015).

Morphospecies	CONVENTIONAL									ORGANIC								
	Vegetative			Reproductive						Vegetative			Reproductive					
	17/12/14	24/12/14	7/1/15	22/1/15	5/2/15	11/2/15	5/3/15	13/3/15	20/3/15	28/11/14	17/12/14	7/1/15	22/1/15	5/2/15	11/2/15	4/3/15	13/3/15	20/3/15
<i>Paridris</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Phanuromyia</i> sp.1	0	0	0	0	2	0	0	0	0	0	0	0	3	0	3	0	1	0
<i>Probaryconus</i> sp.1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Probaryconus</i> sp.2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Scelio</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trimorus</i> sp.1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0
<i>Trimorus</i> sp.2	0	2	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
<i>Trissolcus</i> sp.1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
<i>Trissolcus</i> sp.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trissolcus</i> sp.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Triteleia</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Telenomus</i> sp.1	1	0	11	2	0	0	2	1	0	2	0	3	1	1	2	0	3	0
<i>Telenomus</i> sp.2	0	0	1	0	0	0	0	0	0	0	0	1	2	1	6	1	3	0
<i>Telenomus</i> sp.3	0	0	2	3	0	0	0	1	0	4	0	3	0	0	2	1	1	1
<i>Telenomus</i> sp.4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0
<i>Telenomus</i> sp.5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0
<i>Telenomus podisi</i>	3	0	4	8	0	1	5	12	13	2	1	8	6	3	10	3	2	1
<i>Telenomus</i> sp.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Telenomus</i> sp.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Xenomerus</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	9	4	20	16	2	3	8	16	15	15	3	28	20	9	37	12	21	6