Heliyon 6 (2020) e04634

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Endomycobiome associated with females of the planthopper *Delphacodes kuscheli* (Hemiptera: Delphacidae): A metabarcoding approach

María E. Brentassi [a,b,*], Rocío Medina [c,d], Daniela de la Fuente [a,c], Mario EE. Franco [c,d], Andrea V. Toledo [c,d], Mario CN. Saparrat [c,e,f,g], Pedro A. Balatti [b,d,g]

[a] *División Entomología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina*
[b] *Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Buenos Aires, Argentina*
[c] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[d] *Centro de Investigaciones de Fitopatología (CIDEFI), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires, Argentina*
[e] *Instituto de Fisiología Vegetal (INFIVE), Universidad Nacional de La Plata, Buenos Aires, Argentina*
[f] *Instituto de Botánica Carlos Spegazzini, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina*
[g] *Cátedra de Microbiología Agrícola, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires, Argentina*

ARTICLE INFO

Keywords:
Ecology
Environmental science
Microbiology
Mutualism
Insects
Illumina
Mycobiota
Yeasts

ABSTRACT

A metabarcoding approach was performed aimed at identifying fungi associated with *Delphacodes kuscheli* (Hemiptera: Delphacidae), the main vector of "Mal de Río Cuarto" disease in Argentina. A total of 91 fungal genera were found, and among them, 24 were previously identified for Delphacidae. The detection of fungi that are frequently associated with the phylloplane or are endophytes, as well as their presence in digestive tracts of other insects, suggest that feeding might be an important mechanism of their horizontal transfer in planthoppers. This study draws the baseline for future research regarding mutualistic associations present in *D. kuscheli* as well as their physiological role in the life cycle of this important pest that might lead to developing new management strategies to keep insects populations under control.

1. Introduction

Humans and other animals, such as insects, as well as plants, host a large and ample array of microorganisms that is known as the "microbiota"; the collection of microbial genomes in a host is referred as the "microbiome" (Lederberg and McCray, 2001). These microbial communities interact with their hosts, such a relationship between hosts and their microbial communities enlarge the capacity of organisms to adapt to changing environmental conditions. Fungi are part of this community as the "mycobiota" and constitute the "mycobiome" (Günther et al., 2016; Lederberg and McCray, 2001) that differs among host type, body sites and between individuals. The mycobiome has been studied in animals ranging from ruminants to insects (Bożena et al., 2016).

Fungi and insects inhabit and share ecological niches and frequently they establish a crucial relationship for their success in nature. Regarding such interactions, fungi and within them mostly yeasts, frequently supply insects with sterols, essential vitamins, and many enzymes (Blackwell, 2017a), being outbreeding and spreading the main benefits for fungi (Blackwell, 2017b). Recent studies highlighted that yeasts inhabiting insect's intestines can reside for long periods and mate or generate sexual forms (Blackwell, 2017a; Stefanini, 2018). The digestive tract of insects also hosts pathogens (Marti et al., 2007; Bing et al., 2020) and fungi that are part of a transient microbiome (Boucias et al., 2018).

Among insect fungal associations, those between insects and yeasts have been widely studied due to their effect on both partners (Stefanini, 2018). Yeasts associated with insects include Ascomycota (Saccharomycotina, generally called "true yeast", and Pezizomycotina) as well as a few Basidiomycota. Some members of Pezizomycotina and Basidiomycota have a yeast growth habit and because of this, they are known as yeasts or yeast-like symbiotes (YLS) to distinguish them from Saccharomycotina yeasts (Blackwell, 2017a).

Planthoppers (Hemiptera: Fulgoroidea) are phytophagous insects that feed by inserting their stylets into the vascular tissue of plants sucking phloem sap (Cook and Denno, 1994). Like most plant sap-sucking hemipterans, members of the Delphacidae also are associated with diverse microorganisms including bacterial symbionts (Tang et al., 2010; Urban and Cryan, 2012) and fungi that form part of the mycobiome. This includes yeast-like symbiotes (YLS) that provide a nutritional

* Corresponding author.
E-mail address: eubrenta@fcnym.unlp.edu.ar (M.E. Brentassi).

https://doi.org/10.1016/j.heliyon.2020.e04634
Received 2 March 2020; Received in revised form 9 April 2020; Accepted 3 August 2020

compensation on the restricted diet of these insects that lack some essential nutrients (Noda et al., 1995) and other fungi whose role remains unknown.

The state of the art regarding fungi associated with Delphacidae comes from studies carried out with three major rice planthopper species in Asia: *Sogatella furcifera* (Horváth), *Laodelphax striatellus* (Fallén) and *Nilaparvata lugens* (Stål) and there is only one report in a neotropical rice pest, *Tagosodes orizicolus* (Muir) (Xet-Mull et al., 2004). YLS are obligate symbionts harboured in the mycetocytes formed by fat body cells located in the abdomen (Noda et al., 1995; Xet-Mull et al., 2004), multiply by budding and are vertically transmitted by transovarial infection (Cheng and Hou, 2001; Michalik et al., 2009). Phylogenetic studies indicated that YLS of planthoppers belongs to Pezizomycotina (Noda et al., 1995) that are derived from the entomopathogenic *Cordyceps* clade (Hypocreales: Clavicipitaceae) (Suh et al., 2001). It has been suggested that during evolution these organisms went through considerable changes that were associated with their transition from a filamentous to a budding cell morphology, which additionally changed the relationship with their host, from a pathogenic to an obligate intracellular symbiosis. In planthoppers YLS appeared to play a key role in nitrogen metabolism of the host by recycling uric acid (Sasaki et al., 1996; Hongoh and Ishikawa, 1997), supply the main source for sterol synthesis (ergosta-5,7, 24(28)-trienol) (Wetzel et al., 1992; Noda and Koizum, 2003) and also enzymes for the synthesis of essential amino acids (Xue et al., 2014; Fan et al., 2015; Wan et al., 2016). Genomic studies revealed that YLS contain genes that encode essential steps of biochemical pathways that are missing in the planthopper genome suggesting that YLS might complement the nutritional needs of their host (Xue et al., 2014). Although the mutualistic association with YLS have been widely studied from laboratory-reared planthoppers, other fungi associated are largely unknown. Recent studies by Bing et al. (2020) showed that other fungi related to plants and insects as endophytes or pathogens were also found inside the body of *L. striatellus* and *S. furcifera* collected from rice field populations, however, their role remains unknown.

Delphacodes kuscheli Fennah (Hemiptera: Delphacidae), a species whose generic status is under revision, is a multivoltine species widely distributed in Argentina (latitude 32°–35° S) (Remes Lenicov and Paradell, 2012). This planthopper is a phloem feeder (Brentassi, 2004; Brentassi and Remes Lenicov, 2007; Brentassi et al., 2019) and is the most important natural vector of Mal de Río Cuarto virus (MRCV) (Fijivirus, Reoviridae) (Remes Lenicov et al., 1985). MRCV seriously affects maize (*Zea mays* L.) production in northern and central provinces of Argentina (Lenardón et al., 1998; Giménez Pecci et al., 2012) and also has been detected in Uruguay (Ornaghi et al., 1999). Previous studies of *D. kuscheli* specimens demonstrated that the abdomen of nymphs and adults of both sexes and morphs (macropterous and brachypterous), as well as oocytes, contained YLS, indicating they are vertically transmitted (Brentassi et al., 2014). Also, the number of YLS throughout the host life cycle, from eggs to adults, was quantified showing that in females the number of YLS is higher than in males and also that their number increase during the reproductive period (Liljesthröm et al., 2017). To date, the mycobiota associated with this planthopper pest remains unknown.

Next-generation sequencing (NGS) technologies provided unprecedented opportunities for high-throughput functional genomic research (Morozova and Marra, 2008). In this sense, Illumina technology produces millions of DNA sequence reads in a single run, rapidly changing the landscape of genetic studies (Mardis, 2008). Currently, NGS technologies offer new opportunities for the analysis of the structure and content of microbial genomes (Forde and O'Toole, 2013) and these technologies have been applied to study the structure and species composition of microbial communities associated with insects such as beetles (Miller et al., 2016), ants (Chua et al., 2018), thrips (Kaczmarczyk-Ziemba et al., 2019) and planthoppers (Shentu et al., 2020; Bing et al., 2020).

Considering that the diversity of the microbiota might be affected by factors such as the host's genetic background, physiological condition, sex, and age, as well as the environmental conditions, like temperature, humidity and diet (Boucias et al., 2018; Bing et al., 2018), as a first step towards the study of the endomycobiome associated with *D. kuscheli* we analysed, through a metabarcoding approach, a pool of laboratory-reared females. Our findings will provide information about the endomycobiome associated with *D. kuscheli* as well as the bases for future studies that allow identify the key fungal taxa interacting with this planthopper and their functional contribution in the insect nutrition and life cycle. Such knowledge is crucial to develop a biological management of planthoppers.

2. Materials and methods

2.1. Insects

Planthoppers colonies were grown on oat plants (*Avena sativa* L) in a growth chamber at 24 ± 2 °C, 50–55% relative humidity, and a L16:D8 photoperiod at the Entomology Division (Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata). The founding insects of these colonies (nymphs and adults) were collected in 2012 from Río Cuarto, Córdoba, Argentina, an endemic area for MRCV.

2.2. DNA isolation

One hundred macropterous female adults (2–4 d old) reared under laboratory conditions were selected to include the possible individual variation of the mycobiota composition. Females were surface sterilized by immersing them three times in ethanol (70 % v/v), vortexed and then washed three times with sterile double distilled water following the same procedure used in other studies (Pang et al., 2012; Bing et al., 2020; Shentu et al., 2020). Disinfected insects were pooled in a porcelain mortar, frozen with liquid nitrogen and ground into a fine powder that was resuspended on Nuclei Lysis Solution (at ratio 40 mg of tissue powder/600 μl buffer, Wizard ® Genomic DNA Purification Kit, Promega). Then, the sample was transferred to a microcentrifuge tube, frozen by immersion in liquid nitrogen and incubated at 65 °C for 3 min in a water bath, a procedure that was repeated three times. Then, the sample was vortexed three times and centrifuged for 5 min at 3500 rpm. To check the presence of yeasts in the sample, the supernatant and the resuspended pellet were observed with a microscope. Then, the DNA of the enriched fraction (the pellet) was isolated using the Wizard ® Genomic DNA Purification Kit (Promega) according to the manufacturer instructions. The quality and quantity of DNA were estimated by measuring the optical density (OD) with a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific).

Genomic DNA isolation was confirmed by amplifying the ITS using a pair of fungal primers ITS-4 and ITS-5 (White et al., 1990), according to Franco et al. (2017). Genomic DNA isolated from Ascomycota *Humicolopsis cephalosporioides* strain LPSC 1155 (accession number KY065162), and Basidiomycota *Naganishia diffluens* (accession number MN826140) were included as positive controls of amplification of fungal DNA. Amplified fragments were visualized in 1% agarose gels stained with ethidium bromide that also contained a 100 to 1,000-bp marker (Inbio Highway, Tandil, Argentina). Gels were photo-documented through an image analyzer (Gene Genus analyzer, Syngene) according to Medina et al. (2015).

2.3. Amplicon sequencing

Diversity assay using bTEFAP Illumina MiSeq (2 × 300 PE) was performed at Molecular Research LP (MR DNA, www.mrdnalab.com, Shallowater, TX, USA) using the primer set ITS-5 and ITS-4 (White et al., 1990) and DNA extracted at the previous section as template. Barcode (CACACTCA) was added on 3′ of the forward primer. PCR product was checked in a 2 % (w/v) agarose gel where the presence of the band, as

well as their relative intensity, was determined. The sample was purified using calibrated Ampure XP beads. Then, the purified PCR products were used to prepare the DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed following the manufacturer's guidelines. The sequence data derived from the sequencing process was processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA).

2.4. Data analysis

A low level of joined paired-end reads was observed for the ITS dataset, so the analysis was performed as described by Cobo-Díaz et al. (2019) that described somewhat similar results. Briefly, the forward and reverse files were merged using *multiple_split_libraries_fastq.py*. ITS1 and ITS2 regions were extracted using ITSx v1.0.11 (Bengtsson-Palme et al., 2013) and then were concatenated in a new file. Chimeras were filtered on a concatenated file using UCHIME algorithm (Edgar et al., 2011) with VSEARCH v1.1.3 and UNITE/INSDC (UNITE Community, 2017) as a reference database. Non-chimeric sequences from ITS1-ITS2 concatenated files were used for Operational Taxonomic Units (OTUs) picking running on QIIME, with BLAST as taxonomic assignment method (Altschul et al., 1990), and a non-redundant ITS database version 7.1 of UNITE plus INSD (Koljalg et al., 2013) using as split 3% of the genetic distance using the average-neighbour method. Singletons were removed to minimize the overestimation of rare OTUs. Only OTUs assigned to kingdom Fungi were used for further analysis (Cobo-Díaz et al., 2019).

The quality of the sequencing process was assessed through a rarefaction curve and Good's coverage ($C = 1\text{-}(n_1/N)$, where n_1 is the number of OTUs that have been sampled once and N is the total number of individuals in the sample) were calculated using Mothur v.1.36.1 (Schloss et al., 2009).

3. Results

The ITS amplification of a template DNA extracted from 100 macropterous female adults generated two fragments, 600 and 750 bp long (Fig. S1). Both represent the ITS of organisms that might be living within insects, and probably some contaminants of the sample. As we confirm that extracted DNA was able to use it for ITS amplification, amplicon sequencing was done using this DNA to know the diversity of fungi associated with the insect through bTEFAP Illumina MiSeq technology. Future research using other sequencing methods should be aimed at studying the remaining organisms.

Sequencing provided 134,135 sequences that, after filtering based on quality, algorithms and reference databases as described above, were reduced to 14,683. Among these sequences, 1,457 represented 49 OTUs not belonged to fungi; therefore, they were eliminated, remaining 13,226 fungal sequences represented in 327 OTUs. Raw sequence reads were submitted to the National Center for Biotechnology Information Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) and are available under accession number SRX5076285. The rarefaction curve approached saturation (Fig. S2), and the coverage was higher than 97%, suggesting the high level of performance of the sequencing process.

Sequence analysis and clustering defined a total of 327 fungal OTUs. While 222 OTUs represented 91 genera of fungi the remaining 105 OTUs corresponded to unidentified taxa. The relative abundance as well as the current taxonomic status of the genera according to UNITE plus INSD version 7.1, are presented in Table 1. Among the fungal genera, 29 have already been mentioned in Delphacidae. Seventy-seven per cent of the identified genera belonged to Ascomycota and 23% to Basidiomycota. The abundance of Ascomycota was higher (93.12%) than Basidiomycota (6.14%). The most abundant genera (abundance relative >1) of fungi identified were *Mycosphaerella*, *Didymella*, *Alternaria*, *Penicillium*, *Aspergillus*, *Hannaella*, *Cordyceps*, *Dothistroma*, *Fusarium*, *Nigrospora*, *Stemphylium*, and *Diutina*. Eighteen of the fungal genera identified corresponded to yeasts. Their relative abundance and their current taxonomic status, according to UNITE plus INSD version 7.1, are presented in Table 2. The most represented order within Ascomycota was Saccharomycetales (98.78%) and within Basidiomycota was Tremellales (92.22%) (Figure 1). The relative abundance of genera that belong to Ascomycota and Basidiomycota is shown in Figure 2. While the predominant genera in the ascomycetous group were *Diutina*, *Pichia*, and *Candida*, in the basidiomycetous yeasts were *Hannaella*, *Naganishia*, *Vishniacozyma*, *Sporobolomyces*, and *Rhodotorula*.

Even though the definition of species within organisms should be established based on the ITS as well as additional gene sequences we present here the list of species predicted by the analysis of DNA sequences recovered by the metabarcoding approach assigned by clustering of 97% of genetic similarity (OTUs) (Table S1). Therefore, future studies should be aimed at confirming the identity of the organisms through the whole genome or at least four or five additional gene sequences.

4. Discussion

Currently, researchers emphasize the role that insect's microbiome play in host physiology, behaviour, and evolution (Lewis and Lizé, 2015). Several fungal taxa have been reported as relevant components of insect microbiomes (Parfrey et al., 2018). Fungi, frequently yeasts, are found within different parts of insects (i.e., gut, stomach, hemolymph, fat body, and/or ovary); they might be pathogens, casual passengers or establish a facultative or obligate association providing insects with an advantage in terms of survival and/or nutrition (Gibson and Hunter, 2010; Stefanini, 2018; Vega and Biedermann, 2020).

The present study is the first to provide information through a metabarcoding approach of the mycobiome of *D. kuscheli*. Since the mycobiome might vary between sexes and host's genetic background (Boucias, 2018), we made a preliminary study to know the main components of the endomycobiome of *D. kuscheli* females using a sample that consisted in 100 individuals. Although such an approach prevented us from knowing variations within individuals of a population, overall, this will give us a preliminary idea of all the fungal organisms that might be interacting with *D. kuscheli* at any time. Future studies should be aimed at studying how the environment or the genetic background of the host might affect the composition of the mycobiome as well as its stability.

In this study, 91 fungal genera were found associated with *D. kuscheli* and among them, 24 genera have already been reported in Delphacidae. The genera with a high relative abundance (abundance relative >1) included fungi that were described to be plant pathogens, endophytic, entomopathogens as well as yeasts associated with the plant's phylloplane. *Mycosphaerella, Didymella, Alternaria, Penicillium, Aspergillus*, *Cordyceps, Fusarium*, *Nigrospora*, and *Stemphylium* were reported by several authors as endophytes of tropical plants as well as several grasses (Riesen and Close, 1987; Rubini et al., 2005; Wiyono et al., 2020). *Mycosphaerella* was previously reported as a phytopathogen transmitted and spread by insects (Bergstrom, 1982) and *Didymella* was also found in plant lesions produced by a phytopathogen transmitting insect vector (Park et al., 2018). This observation suggests that most probably such organisms are acquired by *D. kuscheli* when it feeds.

Different genera of fungal entomopathogens have been reported as naturally occurring fungal endophytes (Vega et al., 2008), like a species of *Fusarium* that is a known entomopathogen of the delphacid *Perkinsiella saccharicida* (Kirkaldy) (Rico and Victoria, 1988).

Among the fungi associated with *D. kuscheli* some of the frequent genera found also were identified as dominant fungi of the mycobiome of fertilized and non-fertilized eggs of the planthopper *N. lugens* (Shentu et al., 2020). Also, we found fungi related to plants symbionts and pathogens of plants like *Sarocladium*, *Alternaria*, *Aspergillus,* and *Curvularia* that also have been found within planthoppers collected from rice field populations by Bing et al. (2020). Among them, *Sarocladium* was reported as an entomopathogenic fungus for *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Sharma et al., 2018)

Table 1. Relative abundance of fungal genera recovered from *Delphacodes kuscheli* (Hemiptera: Delphacidae) and their current taxonomic status according to UNITE plus INSD version 7.1. Genera previously reported in Delphacidae are shown in bold.

Genus	Relative abundance	Reads	OTUs	Phylum	Subphylum	Class	Order	Family
Mycosphaerella	21.35	2796	20	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Didymella	10.18	1333	13	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Didymellaceae
Alternaria	**8.92**	**1169**	**13**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Pleosporales**	**Pleosporaceae**
Penicillium	**7.83**	**1025**	**20**	**Ascomycota**	**Pezizomycotina**	**Eurotiomycetes**	**Eurotiales**	**Aspergillaceae**
Aspergillus	**6.21**	**813**	**17**	**Ascomycota**	**Pezizomycotina**	**Eurotiomycetes**	**Eurotiales**	**Aspergillaceae**
Hannaella	4.25	557	3	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae
Cordyceps	**3.21**	**420**	**2**	**Ascomycota**	**Pezizomycotina**	**Sordariomycetes**	**Hypocreales**	**Cordycipitaceae**
Dothistroma	3.01	394	4	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Fusarium	**1.78**	**233**	**2**	**Ascomycota**	**Pezizomycotina**	**Sordariomycetes**	**Hypocreales**	**Nectriaceae**
Nigrospora	1.62	212	6	Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Apiosporaceae
Stemphylium	**1.13**	**148**	**4**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Pleosporales**	**Pleosporaceae**
Diutina	1.10	144	1	Ascomycota	Saccharomycotina	Saccharomycetes	Saccharomycetales	incertae sedis
Wojnowicia	0.97	127	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Phaeosphaeriaceae
Septoria	0.80	105	3	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Naganishia	**0.72**	**94**	**3**	**Basidiomycota**	**Agaricomycotina**	**Tremellomycetes**	**Tremellales**	**Filobasidiaceae**
Curvularia	**0.58**	**76**	**6**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Pleosporales**	**Pleosporaceae**
Gibberella	0.48	63	4	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae
Acremonium	**0.41**	**54**	**4**	**Ascomycota**	**Pezizomycotina**	**Sordariomycetes**	**Hypocreales**	**incertae sedis**
Pichia	**0.37**	**48**	**1**	**Ascomycota**	**Saccharomycotina**	**Saccharomycetes**	**Saccharomycetales**	**Pichiaceae**
Toxicocladosporium	**0.33**	**43**	**1**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Capnodiales**	**Cladosporiaceae**
Leptospora	0.29	38	2	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Phaeosphaeriaceae
Candida	**0.28**	**37**	**4**	**Ascomycota**	**Saccharomycotina**	**Saccharomycetes**	**Saccharomycetales**	**incertae sedis**
Vishniacozyma	0.28	37	3	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae
Cladorrhinum	0.26	34	1	Ascomycota,	Pezizomycotina	Sordariomycetes	Sordariales	Lasiosphaeriaceae
Scytalidium	0.25	33	2	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae
Bipolaris	**0.24**	**31**	**2**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Pleosporales**	**Pleosporaceae**
Readeriella	0.24	31	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Teratosphaeriaceae
Tiarosporella	0.24	31	2	Ascomycota	Pezizomycotina	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae
Dissoconium	0.21	27	4	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Sporobolomyces	**0.19**	**25**	**3**	**Basidiomycota**	**Pucciniomycotina**	**Microbotryomycetes**	**Sporidiobolales**	**Sporidiobolaceae**
Eutypa	0.18	24	1	Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Diatrypaceae
Penidiella	0.18	24	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Teratosphaeriaceae
Beauveria	0.17	22	1	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Cordycipitaceae
Pyrenochaetopsis	0.17	22	2	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pyrenochaetopsidaceae
Phaeothecoidea	0.14	18	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Rhodotorula	**0.12**	**16**	**2**	**Basidiomycota**	**Pucciniomycotina**	**Microbotryomycetes**	**Sporidiobolales**	**Sporidiobolaceae**
Sarocladium	**0.11**	**14**	**1**	**Ascomycota**	**Pezizomycotina**	**Sordariomycetes**	**Hypocreales**	**incertae sedis**
Aureobasidium	**0.10**	**13**	**2**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Dothideales**	**Aureobasidiaceae**
Neodevriesia	0.10	13	3	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Neodevriesiaceae
Periconia	0.09	12	2	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Periconiaceae
Pyrenochaeta	0.09	12	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Cucurbitariaceae
Uwebraunia	0.08	11	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Dissoconiaceae
Catenulostroma	0.08	10	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Teratosphaeriaceae
Papiliotrema	0.08	10	2	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Rhynchogastremataceae
Saitozyma	0.08	10	1	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Trimorphomycetaceae
Malassezia	**0.07**	**9**	**1**	**Basidiomycota**	**Ustilaginomycotina**	**Malasseziomycetes**	**Malasseziales**	**Malasseziaceae**
Xenoramularia	0.07	9	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Bartalinia	0.06	8	1	Ascomycota	Pezizomycotina	Sordariomycetes	Amphisphaeriales	Sporocadaceae
Ascochyta	0.05	7	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Didymellaceae
Botrytis	0.05	7	1	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Sclerotiniaceae
Dioszegia	0.05	7	1	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae
Neoascochyta	0.05	7	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Didymellaceae
Sterigmatomyces	**0.05**	**7**	**1**	**Basidiomycota**	**Pucciniomycotina**	**Agaricostilbomycetes**	**Agaricostilbales**	**Agaricostilbaceae**
Coniosporium	**0.05**	**6**	**2**	**Ascomycota**	**Pezizomycotina**	**Eurotiomycetes**	**Chaetothyriales**	**Herpotrichiellaceae**
Devriesia	0.05	6	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Teratosphaeriaceae
Dicyma	0.05	6	1	Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Xylariaceae
Talaromyces	**0.05**	**6**	**1**	**Ascomycota**	**Pezizomycotina**	**Eurotiomycetes**	**Eurotiales**	**Trichocomaceae**
Auricularia	0.04	5	1	Basidiomycota	Agaricomycotina	Agaricomycetes	Auriculariales	Auriculariaceae

(*continued on next page*)

Table 1 (*continued*)

Genus	Relative abundance	Reads	OTUs	Phylum	Subphylum	Class	Order	Family
Celosporium	0.04	5	1	Ascomycota	Pezizomycotina	Dothideomycetes	Neocelosporiales	Neocelosporiaceae
Cladosporium	**0.04**	**5**	**2**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Capnodiales**	**Cladosporiaceae**
Coniochaeta	0.04	5	1	Ascomycota	Pezizomycotina	Sordariomycetes	Coniochaetales	Coniochaetaceae
Fusariella	0.04	5	1	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	incertae sedis
Martiniozyma	0.04	5	1	Ascomycota	Saccharomycotina	Saccharomycetes	Saccharomycetales	Pichiaceae
Neopestalotiopsis	0.04	5	1	Ascomycota	Pezizomycotina	Sordariomycetes	Amphisphaeriales	Pestalotiopsidaceae
Ramichloridium	0.04	5	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Dissoconiaceae
Cystobasidium	0.03	4	1	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae
Cytospora	0.03	4	1	Ascomycota	Pezizomycotina	Sordariomycetes	Diaporthales	Cytosporaceae
Exobasidium	0.03	4	1	Basidiomycota	Ustilaginomycotina	Exobasidiomycetes	Exobasidiales	Exobasidiaceae
Pleurotus	**0.03**	**4**	**1**	**Basidiomycota**	**Agaricomycotina**	**Agaricomycetes**	**Agaricales**	**Pleurotaceae**
Teratosphaeria	0.03	4	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Teratosphaeriaceae
Symmetrospora	0.03	4	1	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	incertae sedis	Symmetrosporaceae
Acrostalagmus	0.02	3	1	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae
Blumeria	0.02	3	1	Ascomycota	Pezizomycotina	Leotiomycetes	Erysiphales	Erysiphaceae
Cercospora	0.02	3	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Cryptococcus	**0.02**	**3**	**1**	**Basidiomycota**	**Agaricomycotina**	**Tremellomycetes**	**Tremellales**	**Tremellaceae**
Dinemasporium	0.02	3	1	Ascomycota	Pezizomycotina	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae
Kwoniella	0.02	3	1	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Cryptococcaceae
Magnaporthe	0.02	3	1	Ascomycota	Pezizomycotina	Sordariomycetes	Magnaporthales	Magnaporthaceae
Pilatoporus	0.02	3	1	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Fomitopsidaceae
Rachicladosporium	0.02	3	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Cladosporiaceae
Sphaerulina	0.02	3	1	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae
Coriolopsis	0.02	2	1	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Polyporaceae
Graphiola	0.02	2	1	Basidiomycota	Ustilaginomycotina	Exobasidiomycetes	Exobasidiales	Graphiolaceae
Lophodermium	0.02	2	1	Ascomycota	Pezizomycotina	Leotiomycetes	Rhytismatales	Rhytismataceae
Occultifur	0.02	2	1	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae
Phaeosphaeria	0.02	2	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Phaeosphaeriaceae
Poaceascoma	0.02	2	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Lentitheciaceae
Plectosphaerella	0.02	2	1	Ascomycota	Pezizomycotina	Sordariomycetes	Glomerellales	Plectosphaerellaceae
Pseudophaeomoniella	0.02	2	1	Ascomycota	Pezizomycotina	Eurotiomycetes	Phaeomoniellales	Phaeomoniellaceae
Sclerostagonospora	0.02	2	1	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Phaeosphaeriaceae
Thelonectria	0.02	2	1	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae
Unidentified taxa	19.99	2618	105					

Table 2. Relative abundance of yeasts associated with *Delphacodes kuscheli* (Hemiptera: Delphacidae) at the generic level and their taxonomic status according to UNITE plus INSD version 7.1 Genera previously reported in Delphacidae are shown in bold.

Genus	Relative abundance	Reads	OTUs	Phylum	Subphylum	Class	Order	Family
Hannaella	54.13	557	3	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae
Diutina	13.99	144	1	Ascomycota	Saccharomycotina	Saccharomycetes	Saccharomycetales	incertae sedis
Naganishia	**9.13**	**94**	**3**	**Basidiomycota**	**Agaricomycotina**	**Tremellomycetes**	**Tremellales**	**Filobasidiaceae**
Pichia	**4.66**	**48**	**1**	**Ascomycota**	**Saccharomycotina**	**Saccharomycetes**	**Saccharomycetales**	**Pichiaceae**
Candida	**3.59**	**37**	**4**	**Ascomycota**	**Saccharomycotina**	**Saccharomycetes**	**Saccharomycetales**	**incertae sedis**
Vishniacozyma	3.59	37	3	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae
Sporobolomyces	**2.42**	**25**	**3**	**Basidiomycota**	**Pucciniomycotina**	**Microbotryomycetes**	**Sporidiobolales**	**Sporidiobolaceae**
Rhodotorula	**1.55**	**16**	**2**	**Basidiomycota**	**Pucciniomycotina**	**Microbotryomycetes**	**Sporidiobolales**	**Sporidiobolaceae**
Aureobasidium	**1.26**	**13**	**2**	**Ascomycota**	**Pezizomycotina**	**Dothideomycetes**	**Dothideales**	**Aureobasidiaceae**
Papiliotrema	0.97	10	2	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Rhynchogastremataceae
Saitozyma	0.97	10	1	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Trimorphomycetaceae
Malassezia	**0.87**	**9**	**1**	**Basidiomycota**	**Ustilaginomycotina**	**Malasseziomycetes**	**Malasseziales**	**Malasseziaceae**
Dioszegia	0.68	7	1	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae
Sterigmatomyces	**0.68**	**1**	**1**	**Basidiomycota**	**Pucciniomycotina**	**Agaricostilbomycetes**	**Agaricostilbales**	**Agaricostilbaceae**
Kwoniella	0.48	3	1	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Cryptococcaceae
Cystobasidium	0.39	4	1	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae
Martiniozyma	0.29	5	1	Ascomycota	Saccharomycotina	Saccharomycetes	Saccharomycetales	Pichiaceae
Cryptococcus	**0.29**	**3**	**1**	**Basidiomycota**	**Agaricomycotina**	**Tremellomycetes**	**Tremellales**	**Tremellaceae**

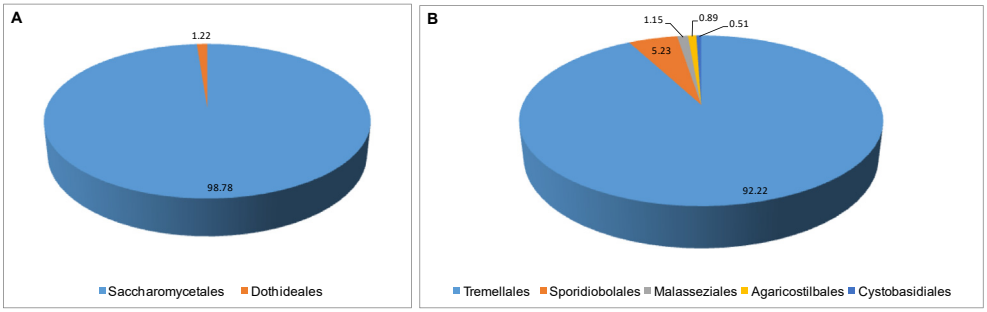

Figure 1. Relative abundance of yeasts associated with *Delphacodes kuscheli* (Hemiptera: Delphacidae) at level of order. A) Ascomycota. B) Basidiomycota.

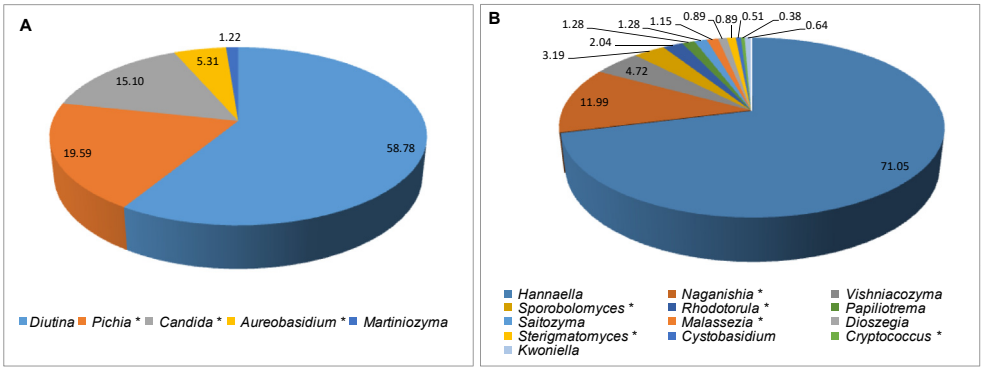

Figure 2. Relative abundance of yeasts associated with *Delphacodes kuscheli* (Hemiptera: Delphacidae) at the generic level. A) Ascomycota. B) Basidiomycota. * indicates genera previously reported in planthoppers belonged to the Delphacidae.

and representatives of the genus *Acremonium* were found living as endophytes of maize and perennial ryegrass (Siegel et al., 1985) and also was reported to be entomopathogens (Vega et al., 2008). Interestingly, one species of *Acremonium* was isolated from the gut of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) by Marti et al. (2007) in what turned out to be one of the few reports describing the presence of entomopathogenic fungi in the mycobiota of the digestive tract. *Acremonium* was mentioned harboured within fat bodies of the delphacid *N. lugens* by Hou et al. (2013). Furthermore, recently *Acremonium implicatum* and *Sarocladium strictum,* were found in eggs of *N. lugens* indicating this that they might be transmitted transovarially (Shentu et al., 2020). However, the presence of *Acremonium* and *Sarocladium* within *D. kuscheli* is not surprising, further studies are necessary to know their specific location, their source of acquisition, as well as the type of relationship they establish with the host and their role in the life cycle of this planthopper.

Another abundant fungus within *D. kuscheli* was *Cordyceps*, an entomopathogenic genus that belongs to Hypocreales. Interestingly, obligate YLS of planthoppers and several groups of plant-sucking hemipterans are closely related to *Cordyceps* (Suh et al., 2001; Nishino et al., 2016; Podsiadło et al., 2018). Further studies are necessary to know the specific role of these fungi in *D. kuscheli.*

Among all the fungi associated whit *D. kuscheli*, we also found several yeasts. Yeasts-insects associations have been widely studied due to their relevant implications for both of the parties involved (Blackwell, 2017a, b). The more abundant genera were *Hannaella* and *Diutina*. Representatives of the genera *Hannaella* have been isolated from environments such as air, phylloplane of several plant species and soil (Wang and Bai, 2008; Landell et al., 2014; Sukmawati et al., 2015) and also were found as part of the gut mycobiota in Lepidoptera pests of maize (Molnár et al., 2008). Similarly, representatives of *Diutina* were isolated from ants and soil (Ba and Phillips, 1996).

In the Delphacidae, YLS have been widely studied by the mutualistic association they establish with their hosts. The dominant YLS previously reported in the Delphacidae belong to Ascomycota (Hypocreales, Clavicipitaceae) (Noda et al., 1995; Suh et al., 2001; Fan et al., 2015), which provide complementary functions to planthoppers such as synthesis of essential amino acids and sterols, nitrogen storage and recycling as well as in carbohydrates metabolism (Xue et al., 2014). Though YLS belonging to Hypocreales have been reported to be dominant endosymbionts of planthoppers, other culturable fungi from the genera *Candida, Cryptococcus, Debaryomyces*, and *Pichia*, as well as uncultured fungi were also found (Dong et al., 2011; Pang et al., 2012; Hou et al., 2013); however, their role remain obscure. Studies by Yu et al. (2014) indicated that YLS of planthoppers are mainly represented by taxa from Sordariomycetes, Saccharomycetes, Tremellomycetes, and Agaricostibomycetes. In this study, we have additionally found representatives of Dothideomycetes, Microbotryomycetes, and Malasseziomycetes, which include genera that also were recently reported in eggs of *N. lugens* (Shentu et al., 2020).

Among the 18 genera of yeasts identified in this study, the genera *Aureobasidium*, *Candida*, *Cryptococcus, Sterigmatomyces*, *Malassezia*, *Naganishia*, *Pichia, Rhodotorula,* and *Sporobolomyces* were previously reported in Delphacidae, and 9 genera are mentioned for the first time within the family. We found that the abundant yeasts in *D. kuscheli* corresponded mostly to Tremellales (*Hannaella* and *Naganishia*), and Saccharomycetales (*Diutina*, *Pichia* and *Candida*). Among Tremellales, *Cryptococcus* sp. also was found in this study. *Cryptococcus*-like symbiotes were found inside fat bodies of the planthopper *N. lugens* (Dong et al., 2011; Yu et al., 2014). The genus *Cryptococcus* was recently redefined (Liu et al. 2015), and representatives of this genus are currently included in the genera *Papiliotrema, Vishniacozyma, Naganishia*, *Saitozyma*, and *Dioszegia.* In this study, several OTUs corresponding to these generic names were detected. *Naganishia* and *Vishniacozyma* representatives were previously isolated from the gut of beetles (Coleoptera: Scolytidae) (Vega and Dowd, 2005; Blackwell, 2017b). Regarding roles of yeast endosymbionts in insects, Sasaki et al. (1996) showed that YLS of *N. lugens* are involved in insect uric acid metabolism mobilizing its reserve as a source of nitrogen for the synthesis of amino acids improving in this way

the host nutrition. Interestingly, Vera-Ponce de León et al. (2016) isolated different species of *Cryptococcus*, as well as *Rhodotorula*, *Debaryomyces*, *Trametes*, and *Penicillium* from *Dactylopius* (Hemiptera: Coccoidea: Dactylopiidae), whose genomes contain genes that code proteins involved in uric acid catabolism, suggesting that they probably help insects to recycle nitrogen. Interestingly *Rhodotorula mucilaginosa* was previously mentioned as an uricolytic fungal symbiont of *Dactylopius* spp. (Hemiptera: Dactylopiidae) by Vera-Ponce de León et al. (2013). Based on previous findings and on the fact that fungi previously identified as *Cryptococcus* as well as *Rhodotorula* species were found associated with *D. kuscheli*, further studies should evaluate the role these fungi might play in recycling nitrogenous compounds in this planthopper pest.

Among Saccharomycetales the most abundant genera associated with *D. kuscheli* were *Diutina*, *Pichia,* and *Candida*. *Pichia*-like and *Candida*-like symbiotes were previously reported as YLS in species of planthoppers (Eya et al., 1989; Dong et al., 2011; Hughes et al., 2011; Pang et al., 2012; Hou et al., 2013; Wang et al., 2014; Yu et al., 2014; Cao et al., 2015). Several fungi tentatively identified as taxa corresponding to the genus *Candida* also have been reported in several orders of insects (Vega and Dowd, 2005) and were frequently isolated from guts of honey bees (Gilliam et al., 1974), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), some families of Neuroptera and Blattodea (Nguyen et al., 2007) as well as from the digestive tract of the mealybug *Pseudococcus longispinus* (Targioni--Tozzetti) (Hemiptera: Pseudococcidae) (Popova et al., 2016). Previous studies by Eya et al. (1989) showed that in the planthopper *L. striatellus,* a YLS member identified as *Candida* sp. produced ergosterol and provided it to the host as a source of 24-methylenecholesterol and cholesterol. Interestingly, *C. parapsilosis* can synthesize sterols, primarily ergosterol and zymosterol (Ba et al., 1995; Ba and Phillips, 1996) suggesting a probable role for supplying ergosterol to insects.

Several yeasts associated with *D. kuscheli*, such as those of the following genera: *Vishniacozyma, Dioszegia*, *Rhodotorula, Sporobolomyces* and *Aureobasidium,* also have been found within the mycobiota of phylloplane (Fonseca and Inácio, 2006; Kemler et al., 2017).

The presence in *D. kuscheli* of fungi frequently reported as endophytic and associated with the phylloplane and digestive tract of insects, suggests that the feeding behaviour might be one of the ways that this planthopper acquires at least several of these fungi. *Delphacodes kuscheli* repeatedly explores the plant surface with the labium and secretes a small amount of saliva to dissolve and test chemical components, which is followed by extensive periods of salivation and ingestion in phloem sap (Brentassi, 2004; Brentassi et al., 2019). This behaviour may lead to the ingestion of fungi that inhabit the phylloplane as well as fungal endophytes associated with their gramineous host plants. So, since the feeding behaviour appears to be a way fungi reach the digestive tract, this might be the most relevant mechanism of horizontal transmission of YSL in planthoppers. In this sense, it is interesting to highlight that horizontal symbiotes transfer has been considered a mechanism that might explain the close phylogenetic relationship between the aphid and planthopper YLS (Fukatsu and Ishikawa, 1996). Additionally, recent studies highlighted that entomopathogenic fungi can infect insects via oral ingestion (Mannino et al., 2019), which is another evidence supporting that ingestion might be a possible way of acquisition of fungal pathogens and symbionts by *D. kuscheli*. In line with this Bing et al. (2020) demonstrated that the guts of two planthoppers pests harboured more fungi than other tissues and suggested the possibility that fungi were acquired from direct feeding.

In summary, our results increase the knowledge regarding the diversity of fungi present in the Delphacidae and analyse for the first time the endomycobiome associated with *D. kuscheli*. Since several fungi associated with *D. kuscheli* are frequent inhabitants of the phylloplane and some are endophytes, we suggest that feeding might be one of the possible mechanisms of horizontal transfer in planthoppers. This work intends to be the starting point for further exploring the nature of the ecological relationship between *D. kuscheli* and the dominant representatives of its mycobiome as well as their specific functions that might impact the life cycle of this pest, what might lead to the development of new management strategies.

Declarations

Author contribution statement

María E Brentassi, Rocío Medina: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Daniela de la Fuente: Contributed reagents, materials, analysis tools or data.

Mario EE Franco: Conceived and designed the experiments; Analyzed and interpreted the data.

Andrea V Toledo, Pedro A Balatti: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mario CN Saparrat: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by grants from Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de La Plata (UNLP) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCy) (PICT, 2015–2349).

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04634.

Acknowledgements

DdlF is fellow of CONICET; MEB and PAB are researchers of CIC and AVT, RM, MEEF, and MCNS are researchers of CONICET. We would like to thank José Francisco Cobo-Díaz and Antonio José Fernández González for their great collaboration and willingness to help us carry out the bioinformatic analysis.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Ba, A.S., Guo, D.A., Norton, R.A., Phillips, S.A., Nes, W.D., 1995. Developmental differences in the sterol composition of *Solenopsis invicta*. Arch. Insect Biochem. Physiol. 29, 1–9.

Ba, A.S., Phillips, S.A., 1996. Yeast biota of the red imported fire ant. Mycol. Res. 100, 740–746.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., et al., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919.

Bergstrom, G.C., 1982. Role of insect injury and powdery mildew in the epidemiology of the gummy stem blight disease of cucurbits. Plant Dis. 66, 683–686.

Bing, X., Gerlach, J., Loeb, G., Buchon, N., 2018. Nutrient-dependent impact of microbes on *Drosophila suzukii* development. mBio 9 e02199-17.

Bing, X.L., Zhao, Dian-Shu, Peng, Chang-Wu, Huang, Hai-Jian, Hong, Xiao-Yue, 2020. Similarities and spatial variations of bacterial and fungal communities in field rice planthopper (Hemiptera: Delphacidae) populations. Insect Sci.

Blackwell, M., 2017a. Made for each other: ascomycete yeasts and insects. Microbiol. Spectr. 5 (3).

Blackwell, M., 2017b. Yeasts in insects and other invertebrates. In: Buzzini, P., Lachance, M.A., Yurkov, A. (Eds.), Yeasts in Natural Ecosystems: Diversity. Springer, Heidelberg, pp. 397–433.

Bożena, D.K., Iwona, D., Ilona, K., 2016. The mycobiome - a friendly cross-talk between fungal colonizers and their host. Ann. Parasitol. 62 (3), 175–184.

Boucias, D.G., Zhou, Y., Huang, S., Keyhani, N.O., 2018. Microbiota in insect fungal pathology. Appl. Microbiol. Biotechnol. 102 (14), 5873–5888.
Brentassi, M.E., 2004. Estudio de la interacción planta-insecto. Comportamiento alimentario del vector del Mal de Río Cuarto del maíz, *Delphacodes kuscheli* Fennah (Insecta-Hemiptera-Delphacidae). Doctoral thesis, Universidad Nacional de La Plata, Argentina, p. 145pp.
Brentassi, M.E., de la Fuente, D., Lameiro, A., 2014. Localización y caracterización morfológica de endosimbiontes obligados de *Delphacodes kuscheli* y *Dalbulus maidis*, dos especies de chicharritas vectoras de enfermedades del cultivo de maíz en Argentina (Hemiptera: auchenorryncha). Rev Cs Morfol 16, 1–7.
Brentassi, M.E., Remes Lenicov, A.M., 2007. Feeding behaviour of the vector *Delphacodes kuscheli* (Hemiptera: fulgoromorpha: Delphacidae) on maize and oat. Ann. Soc. Entomol. Fr. 43, 205–212.
Brentassi, M.E., Machado-Assefh, C.R., Alvarez, A.E., 2019. The probing behaviour of the planthopper *Delphacodes kuscheli* (Hemiptera: Delphacidae) on two alternating hosts, maize and oat. Aust. Entomol. 58, 666–674.
Cao, W., Ma, Z., Chen, Y.H., Yu, X., 2015. *Pichia anomala*, a new species of yeast-like endosymbionts and its variation in small brown planthopper (*Laodelphax striatellus*). J. Biosci. Bioeng. 119, 669–673.
Cheng, D.J., Hou, R.F., 2001. Histological observations on transovarial transmission of a yeast-like symbiote in *Nilaparvata lugens* Stål (Homoptera, Delphacidae). Tissue Cell 33, 273–279.
Chua, K.O., Song, S.L., Yong, H.S., See-Too, W.S., Yin, W.F., Chan, K.G., 2018. Microbial community composition reveals spatial variation and distinctive core microbiome of the weaver ant *Oecophylla smaragdina* in Malaysia. Sci. Rep. 8, 10777.
Cobo-Díaz, J.F., Baroncelli, R., Le Floch, G., Picot, A., 2019. Combined metabarcoding and co-occurrence network analysis to profile the bacterial, fungal and *Fusarium* communities and their interactions in maize stalks. Front. Microbiol. 10, 261.
Cook, A.G., Denno, R.F., 1994. Plant interactions: feeding behaviour, plant nutrition, plant defense, and host plant specialization. In: Denno, R.F., Perfect, T.J. (Eds.), Planthoppers. Springer, Verlag, Berlin Heidelberg, pp. 114–139.
Dong, S., Pang, K., Bai, X., Yu, X., Hao, P., 2011. Identification of two species of yeast-like symbiotes in the brown planthopper, *Nilaparvata lugens*. Curr. Microbiol. 62, 1133–1138.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Eya, B.K., Kenny, P.T.M., Tamura, S.Y., Ohnishi, M., Naya, Y., Nakanishi, K., Sugiura, M., 1989. Chemical association in symbiosis: sterol donor in planthoppers. J. Chem. Ecol. 15, 373–380.
Fan, H.W., Noda, H., Xie, H.Q., Suetsugu, Y., Zhu, Q.H., Zhang, C.X., 2015. Genomic analysis of an Ascomycete fungus from the rice planthopper reveals how it adapts to an endosymbiotic lifestyle. Genome Biol. Evol 7, 2623–2634.
Fonseca, Á., Inácio, J., 2006. Phylloplane yeasts. In: Rosa, C.A., Péter, G. (Eds.), Biodiversity and Ecophysiology of Yeasts. The Yeast Handbook. Springer, Berlin Heidelberg, pp. 263–301.
Forde, B.M., O'Toole, P.W., 2013. Next-generation sequencing technologies and their impact on microbial genomics. Brief Funct Genomics 12, 440–453.
Franco, M.E.E., Troncozo, M.I., López, S.M.Y., Lucentini, G., Medina, R., Saparrat, M.C.N., Ronco, L.B., Balatti, P.A., 2017. A survey on tomato leaf grey spot in the two main production areas of Argentina led to the isolation of *Stemphylium lycopersici* representatives which were genetically diverse and differed in their virulence. Eur. J. Plant Pathol. 149, 983–1000.
Fukatsu, T., Ishikawa, H., 1996. Phylogenetic position of yeast-like symbiont of *Hamiltonaphis styraci* (Homoptera, Aphididae) based on 18S rDNA sequence. Insect Biochem. Mol. Biol. 26, 383–388.
Gilliam, M., Wickerham, L.J., Morton, H.L., Martin, R.D., 1974. Yeasts isolated from honey bees, *Apis mellifera*, fed 2,4-D and antibiotics. J. Invertebr. Pathol. 24, 349–356.
Giménez Pecci, M.P., Laguna, I.G., Lenardón, S.L., 2012. Mal de Río Cuarto del maíz. In: Giménez Pecci, M.P., Laguna, I.G., Lenardón, S.L. (Eds.), Enfermedades del maíz producidas por virus y mollicutes en Argentina. INTA, Buenos Aires, Argentina, pp. 41–56.
Gibson, CM, Hunter, MS, 2010. Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. Ecol. Lett. 13 (2), 223–224.
Günther, C., Josenhans, C., JanWehkamp, J., 2016. Crosstalk between microbiota, pathogens and the innate immune responses. Int. J. Med. Microbiol.
Hongoh, Y., Ishikawa, H., 1997. Uric acid as a nitrogen resource for the brown planthopper, *Nilaparvata lugens*: studies with synthetic diets and aposymbiotic insects. Zool. Sci. 14, 581–586.
Hou, Y., Ma, Z., Dong, S., Chen, Y.H., Yu, X., 2013. Analysis of yeast-like symbiote diversity in the brown planthopper (bph), *Nilaparvata lugens* Stål, using a novel nested PCR-DGGE protocol. Curr. Microbiol. 67, 263–270.
Hughes, G.L., Allsopp, P.G., Webb, R.I., Yamada, R., Iturbe-Ormaetxe, I., Brumbley, S.M., O'Neill, S.L., 2011. Identification of yeast associated with the planthopper, *Perkinsiella saccharicida*: potential applications for Fiji leaf gall control. Curr. Microbiol. 63, 392–401.
Kaczmarczyk-Ziemba, A., Wagner, G.K., Grzywnowicz, K., Kucharczyk, M., Zielińska, S., 2019. The microbiome profiling of fungivorous black tinder fungus beetle *Bolitophagus reticulatus* reveals the insight into bacterial communities associated with larvae and adults. PeerJ 7, e6852.
Kemler, M., Witfel, F., Begerow, D., Yurkov, A., 2017. Phylloplane yeasts in temperate climates. In: Buzzini, P., Lachance, M.A., Yurkov, A. (Eds.), Yeasts in Natural Ecosystems: Diversity. Springer, Heidelberg, pp. 171–197.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Landell, M.F., Brandão, L.R., Barbosa, A.C., Ramos, J.P., Safar, S.V.B., Gomes, F.C.O., Sousa, F.M.P., Morais, P.B., Broetto, L., Leoncini, O., et al., 2014. *Hannaella pagnoccae* sp. nov., a tremellaceous yeast species isolated from plants and soil. Int. J. Syst. Evol. Microbiol. 64, 1970–1977.
Lederberg, J., McCray, A.T., 2001. 'Ome Sweet 'Omics – a genealogical treasury of words. Scientist 15, 8.
Lenardón, S.L., March, G.J., Nome, S.F., Ornaghi, J.A., 1998. Recient outbreak of Mal de Río Cuarto virus on corn in Argentina. Plant Dis. 82, 448.
Lewis, Z., Lizé, A., 2015. Insect behaviour and the microbiome. Curr. Opinion Insect Sci. 9, 86–90.
Liljesthröm, G., Brentassi, M.E., Remes Lenicov, A.M., 2017. Modeling population dynamics of yeast-like symbionts (Ascomycota: pyrenomycetes: Clavicipitaceae) of the planthopper *Delphacodes kuscheli* (Hemiptera: Delphacidae). Symbiosis 72, 171–181.
Liu, X.Z., Wang, Q.M., Göker, M., Groenewald, M., Kachalkin, A.V., Lumbsch, H.T., Millanes, A.M., Wedin, M., Yurkov, A.M., Boekhout, T., Bai, F.Y., 2015. Towards an integrated phylogenetic classification of the Tremellomycetes. Stud. Mycol. 81, 85–147.
Mannino, M.C., Huarte-Bonnet, C., Davyt-Colo, B., Pedrini, N., 2019. Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. J. Fungi 5 (2) pii: E33.
Mardis, E.R., 2008. The impact of next-generation sequencing technology on genetics. Trends Genet. 24, 133–141.
Marti, G.A., García, J.J., Cazau, M.C., López Lastra, C.C., 2007. Fungal flora of the digestive tract of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina. B Soc Argent Bot 42, 175–179.
Medina, R., López, S.M., Franco, M.E., Rollan, C., Ronco, B.L., Saparrat, M.C.N., De Wit, P., Balatti, P.A., 2015. A survey on occurrence of *Cladosporium fulvum* identifies race 0 and race 2 in tomato-growing areas of Argentina. Plant Dis. 99, 1732–1737.
Molnár, O, Wuczkowski, M, Prillinger, H, 2008. Yeast biodiversity in the guts of several pests on maize; comparison of three methods: classical isolation, cloning and DGGE. Mycol. Progress 7, 111–123.
Michalik, A., Jankowska, W., Szklarzewicz, T., 2009. Ultrastructure and transovarial transmission of endosymbiotic microorganisms in *Conomelus anceps* and *Metcalfa pruinosa* (Insecta: Hemiptera: fulgoromorpha). Folia Biol. 57, 131–137.
Miller, K.E., Hopkins, K., Inward, D.J., Vogler, A.P., 2016. Metabarcoding of fungal communities associated with bark beetles. Ecol. Evol. 6, 1590–1600.
Morozova, O., Marra, M.A., 2008. Applications of next-generation sequencing technologies in functional genomics. Genomics 92, 255–264.
Nishino, T., Tanahashi, M., Lin, C.P., Koga, R., Fukatsu, T., 2016. Fungal and bacterial endosymbionts of eared leafhoppers of the subfamily Ledrinae (Hemiptera: Cicadellidae). Appl. Entomol. Zool. 51, 465–477.
Nguyen, N.H., Suh, S.O., Blackwell, M., 2007. Five novel *Candida* species in insect-associated yeast clades isolated from Neuroptera and other insects. Mycologia 99, 842–858.
Noda, H., Nakashima, N., Koizumi, M., 1995. Phylogenetic position of yeast like symbiotes of rice planthoppers based on partial 18S rDNA sequences. Insect Biochem. Mol. Biol. 25, 639–646.
Noda, H., Koizum, Y., 2003. Sterol biosynthesis by symbiotes: cytochrome P450 sterol C-22 desaturase genes from yeast like symbiotes of rice planthoppers and anobiid beetles. Insect Biochem. Mol. Biol. 33, 649–658.
Ornaghi, J., Beviacqua, J.E., Aguirrezabala, D.A., March, G.J., Lenardón, S.L., 1999. Detection of mal de Río Cuarto virus in Uruguay. Braz Phytopatol. 24, 471.
Pang, K., Dong, S.Z., Hou, Y., Bian, Y.L., Yang, K., Yu, X.P., 2012. Cultivation, identification and quantification of one species of yeast-like symbionts, *Candida*, in the rice brown planthopper, *Nilaparvata lugens* Stål. Insect Sci. 19, 477–484.
Parfrey, L.W., Moreau, C.S., Russell, J.A., 2018. Introduction: the host-associated microbiome: pattern, process and function. Mol. Ecol. 27, 1749–1765.
Park, J.M., You, Y., Back, C., et al., 2018. Fungal load in *Bradysia agrestis*, a phytopathogen-transmitting insect vector. Symbiosis 74, 145–158.
Popova, T.P., Trencheva, K.G., Tomov, R.I., 2016. Investigation on the microflora of the longtailed mealybug *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae) in order to assess its importance as a carrier of pathogenic microorganisms. Bulg J. Agric. Sci. 22, 103–107.
Podsiadło, E., Michalik, K., Michalik, A., Szklarzewiczet, T., 2018. Yeast-like microorganisms in the scale insect *Kermes quercus* (Insecta, Hemiptera,Coccomorpha: kermesidae). Newly acquired symbionts? Arthropod Struct. Dev. 47, 56–63.
Remes Lenicov, A.M., Tesón, A., Dagoberto, E., Huguet, N., 1985. Hallazgo de uno de los vectores del "Mal de Río Cuarto en maíz". Gaceta Agropecuaria VXXV, 251–258.
Remes Lenicov, A.M., Paradell, S., 2012. Morfología y biología de especies vectoras de virus y mollicutes al maíz en la Argentina (Insecta-Hemiptera: Cicadomorpha-Fulgoromorpha). In: Giménez Pecci, M.P., Laguna, G. (Eds.), Enfermedades del maíz producidas por virus y mollicutes en Argentina. INTA Buenos Aires, Argentina, pp. 82–101.
Rico, J., Victoria, J.I., 1988. Evaluación e identificación de microorganismos patógenos de *Perkinsiella saccharicida* (Hom: delphacidae) en caña de azúcar. Acta Agron. 38, 31–40.
Riesen, T.K., Close, R.C., 1987. Endophytic fungi in propiconazole-treated and untreated barley leaves. Mycologia 79, 546–552.
Rubini, M.R., Silva-Ribeiro, R.T., Pomella, A.W., Maki, C.S., Araújo, W.L., Santos, D.R., Azevedo, J.L., 2005. Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. Int. J. Biol. Sci. 1, 24–33.

Sasaki, T., Kawamura, M., Ishikawa, H., 1996. Nitrogen recycling in the brown planthopper, *Nilaparvata lugens*: Involvement of yeast-like endosymbionts in uric acid metabolism. J. Insect Physiol. 42, 125–129.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Sharma, L., Gonçalves, F., Oliveira, I., Torres, L., Marques, G., 2018. Insect-associated fungi from naturally mycosed vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). Biocontrol Sci. Technol. 28, 122–141.

Shentu, X., Xiao, Y., Song, Y., Cao, Z., Fan, J., Yu, X., 2020. Comparative analysis of the diversity of the microbial communities between non-fertilized and fertilized eggs of brown planthopper. *Nilaparvata lugens* Stål Insects 11, 49.

Siegel, M.C., Latch, G.C.M., Johnson, M.C., 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: significance and control. Plant Dis. 69, 79–83.

Stefanini, I., 2018. Yeast-insect associations: it takes guts. Yeast 35, 315–330.

Suh, S.O., Noda, H., Blackwell, M., 2001. Insect Symbiosis: derivation of yeast-like endosymbionts within an entomopathogenic filamentous lineage. Mol. Biol. Evol. 18, 995–1000.

Sukmawati, D., Oetari, A., Hendrayanti, D., Atria, M., Sjamsuridzal, W., 2015. Identification of phylloplane yeasts from paper mulberry (*Broussonetia papyrifera* (L.) L'Hér. ex Vent.) in Java, Indonesia. Malays J. Microbiol. 11, 324–340.

Tang, M., Lv, L., Jing, S., Zhu, L., He, G., 2010. Bacterial symbionts of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). Appl. Environ. Microbiol. 76 (6), 1740–1745.

UNITE Community, 2017. UNITE USEARCH/UTAX release. Available at: https://search.datacite.org/works/10.15156/bio/587476.

Urban, J.M., Cryan, J.R., 2012. Two ancient bacterial endosymbionts have coevolved with the planthoppers (Insecta: Hemiptera: Fulgoroidea). BMC Evol. Biol. 12, 87. http://www.biomedcentral.com/1471-2148/12/87.

Vega, F.E., Dowd, P.F., 2005. The role of yeasts as insect endosymbionts. In: Vega, F.E., Blackwell, M. (Eds.), Insect-Fungal Associations: Ecology and Evolution. Oxford University Press Publisher, New York, pp. 211–243.

Vega, F.E., Posada, F., Aimed, M.C., Pava-Ripoll, M., Infante, F., Rehner, S.A., 2008. Entomopathogenic fungal endophytes. Biol. Contr. 46, 72–82.

Vega, F.E., Biedermann, P.H.W., 2020. On Interactions, Associations, Mycetangia, Mutualists and Symbiotes in Insect-Fungus Symbioses Fungal Ecology, p. 44.

Vera-Ponce de León, A., Rosenblueth, M., Martínez-Romero, E., 2013. Uricolityc fungi symbionts of nopal cochineal *Dactylopius* spp. (Hemiptera: Dactylopiidae). In: Conference: Entomological Society of America Annual Meeting.

Vera-Ponce de León, A., Sanchez-Flores, A., Rosenblueth, M., Martínez-Romero, E., 2016. Fungal community associated with *Dactylopius* (Hemiptera: Coccoidea: Dactylopiidae) and its role in uric acid metabolism. Front. Microbiol. 7, 954.

Wan, P.J., Tang, Y.H., Yuan, S.Y., Wang, W.X., Lai, F.X., Yu, X., Fu, Q., 2016. ATP phosphoribosyltransferase from symbiont *Entomomyces delphacidicola* involved in histidine biosynthesis of *Nilaparvata lugens* (Stål). Amino Acids 48, 2605–2617.

Wang, Q.M., Bai, F.Y., 2008. Molecular phylogeny of basidiomycetous yeasts in the *Cryptococcus luteolus* lineage (Tremellales) based on nuclear rRNA and mitochondrial cytochrome b gene sequence analyses: proposal of *Derxomyces* gen. nov. and *Hannaella* gen. nov., and description of eight novel *Derxomyces* species. FEMS Yeast Res. 8, 799–814.

Wang, Y., Wang, Y., Zhang, X., Chen, M., Huang, B., 2014. Endosymbionts diversity in two planthoppers estimated by 28S rDNA analysis. J. Anhui Agric. Univ. 41, 768–771.

Wetzel, J.M., Ohnishi, M., Fujita, T., Nakanishi, K., Naya, Y., Noda, H., Sugiura, M., 1992. Diversity in steroidogenesis of symbiotic microorganisms from planthoppers. J. Chem. Ecol. 18, 2083–2094.

White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315–322.

Wiyono, S., Prakoso, B.B., Santoso, S., 2020. Endophytic fungi play important role in rice protection against brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) Conf. Series. Earth Environ. Sci. 468, 012047. IOP Publishing.

Xet-Mull, A.M., Quesada, T., Espinoza, A.M., 2004. Phylogenetic position of the yeast-like symbiotes of *Tagosodes orizicolus* (Homoptera: Delphacidae) based on 18S ribosomal DNA partial sequences. Rev. Biol. Trop. 52, 777–785.

Xue, J., Zhou, X., Zhang, C.X., Yu, L.L., Fan, H.W., Wang, Z., Xu, H.J., Xi, Y., Zhu, Z.-R., Zhou, W.W., Pan, P.L., Li, B.L., Colbourne, J.K., Noda, H., Suetsugu, Y., Kobayashi, T., Zheng, Y., Liu, S., Zhang, R., Liu, Y., Luo, Y.D., Fang, D.M., ChenY, Zhan, D.L., Lv, X.D., Cai, Y., Wang, Z.B., Huang, H.J., Cheng, R.L., Zhang, X.C., Lou, Y.H., Yu, B., Zhuo, J.C., Ye, Y.X., Zhang, W.Q., Shen, Z.C., Yang, H.M., Wang, J., Wang, J., Bao, Y.Y., Cheng, J.A., 2014. Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. Genome Biol. 15, 521.

Yu, H., Ji, R., Ye, W., Chen, H., Lai, W., Fu, Q., Lou, Y., 2014. Transcriptome analysis of fat bodies from two brown planthopper (*Nilaparvata lugens*) populations with different virulence levels in rice. PloS One 9 (2), e88528.