Meta-transcriptomic analysis of the virome and microbiome of the invasive Indian myna (Acridotheres tristis) in Australia

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\textbf{ABSTRACT}

Invasive species exert a serious impact on native fauna and flora and have become the target of eradication and management efforts worldwide. Invasive avian species can also be important pathogen reservoirs, although their viromes and microbiomes have rarely been studied. As one of the top 100 invasive pest species globally, the expansion of Indian mynas (Acridotheres tristis) into peri-urban and rural environments, in conjunction with increasing free-ranging avian agricultural practices, may increase the risk of microbial pathogens jumping species boundaries. Herein, we used a meta-transcriptomic approach to explore the microbes present in brain, liver and large intestine of 16 invasive Indian myna birds in Sydney, Australia. From this, we discovered seven novel viruses from the families Adenoviridae, Caliciviridae, Flaviviridae, Parvoviridae and Picornaviridae. Interestingly, each of the novel viruses identified shared less than 80% genomic similarity with their closest relatives from other avian species, indicative of a lack of detectable virus transmission between invasive mynas to native or domestic species. Of note, we also identified two coccidian protozoa, Isospora superbus and Isospora greineri, from the liver and gut tissues of mynas. Overall, these data demonstrate that invasive mynas can harbor a diversity of viruses and other microorganisms such that ongoing pathogen surveillance in this species is warranted.

1. Introduction

Originally native to southeast Asia, the Indian (common) myna bird (Acridotheres tristis) was introduced into Australia to control insects in market gardens during the late 1800s following the expansion of agriculture and urban development. These animals are omnivorous, capable of exploiting diverse landscapes and are aggressive to other birds and small mammals, driving their rapid spread throughout Australia and abroad. As a consequence mynas are now classified as one of the top 100 invasive pest species globally [1]. Many invasive species are highly adaptable, competing for food, roosting and nesting resources with native Australian species [2]. Since their population is now widely distributed and rapidly expanding both in Australia and globally, the ecological significance of invasive mynas in Australia makes them attractive to study from the perspective of population dynamics, biology, aggressive behavior, and as potential vectors for known and novel infectious agents. For example, the Indian myna will spread the seeds of agricultural pest weeds and damage ripening fruits [3]. Invasive mynas have also shown a capacity to carry pathogens such as Trichomonas gallinae and Haemoporphidarian parasites (Plasmodium and related Haemoproteus spp.), [4] Salmonella sp. and mites [5]. Although a previous study detected avian siadenovirus [6] and low pathogenic avian influenza [7] in free-living mynas, little is known about the clinical and zoonotic risks of any viruses these animals may carry.

Invasive and native birds can function as reservoirs for a variety of important human and veterinary pathogens, such as members of the family Flaviviridae (positive-sense RNA viruses), including avian-associated flaviviruses that infect domestic animals and cause considerable socio-economic impacts on public health and the agricultural industries [8]. Notably, two related genera of the Flaviviridae - hepacviruses and pegviruses - have been detected in an increasingly wide array of species, including Australian wildlife, providing a new perspective on the diversity of this important group of viruses [9]. For example, pegviruses have been identified in a variety of mammalian hosts, including human and non-human primates, bats, horses, rodents, cetaceans [10] and more recently geese [11] and felines [12]. Infections...
asymptomatic infection and/or presenting as diseases such as gastroenteritis, avian encephalomyelitis and hemorrhagic hepatitis in a range of host species [15, 16]. Similarly, avian picornaviruses (Picornaviridae; positive-sense RNA viruses) can cause a reduction of egg production, catarhal enteritis, avian encephalomyelitis and hemorrhagic hepatitis in a range of waterfowl and poultry, and have been associated with avian keratinocytic disorders (AKD) and beak deformity in wild birds [17]. Collectively, picornaviruses exhibit tremendous phylogenetic diversity and a wide host range, and some have the potential to contribute to economic loss and negative impacts on wildlife conservation.

Metagenomic sequencing has greatly increased our understanding of viral diversity and cryptic pathogens in wildlife [18]. For example, metagenomic studies of seemingly healthy avian species have revealed a diverse array of novel avian picornaviruses and caliciviruses from ducks, penguins, cranes and lorikeets [19] with no clear disease association. Importantly, characterizing the virome of highly invasive species through metagenomics can provide a better understanding of the potential ecological consequences and microbial interplay between introduced and native species in an urban bird community [25]. Herein, we used meta-transcriptomics to reveal the diversity of viruses and other microbes in apparently healthy mynas in Australia, with the specific aim of documenting any that may have transmitted to or from native bird species.

2. Methods

2.1. Sample collection

Sixteen Indian (common) mynas (A. tristis) were captured from a suburban environment in North Sydney, NSW, Australia in early 2019. The mynas used in this study were trapped and euthanised during routine community pest control. Birds were trapped in wire cages, transported less than 30 min, euthanised with carbon dioxide and immediately subjected to gross post-mortem examination and sample collection. After necropsy, the sample tissues were preserved in RNA-later® and stored under −80°C prior to RNA extraction. All methods were approved by Taronga Conservation Society Australia’s Animal Ethics Committee (approval number 3B1218) in compliance with the Australian Code of Practice for the Use of Animals for Scientific Purposes.

2.2. RNA library construction and metatranscriptomic sequencing

RNA was extracted from samples of brain, liver and large intestine from the 16 birds using the RNeasy Plus mini kit (Qiagen, Hilden, Germany) then pooled into six libraries based on these tissue types. RNA concentration and integrity were determined using a Nanodrop spectrophotometer (ThermoFisher, MA, USA) and TapeStation (Agilent, CA, USA). RNA samples were pooled in equal proportions based on animal tissue type. Illumina RNA libraries were then prepared from the pooled samples following RNA depletion using a RiboZero Gold kit (Epigene, Cambridge, MA) then sequenced on an Illumina NovaSeq platform (paired 150-nt reads). Raw sequencing reads were quality trimmed with Trimmomatic [26] then de novo assembled using Megahit [27]. To identify potential virus-like transcripts, sequence reads were assembled into contigs and blasted against the NCBI non-redundant protein (nr) database with searches using Diamond blastx at an e-value cut-off E−5 [28]. All viral reads were then reassembled to obtain a complete virus genome using the Geneious Assembler program implemented in Geneious Prime 2021.1.1. Putative cleavage sites and motifs were identified through comparative genomic analysis.

2.3. Phylogenetic analysis of the novel viruses

A diverse set of nucleotide and amino acid sequences, including the RNA-dependent RNA polymerase (RdRp) that is conserved among RNA viruses, were downloaded from GenBank and employed as background reference sequences in phylogenetic analyses. Accordingly, the putative NS3 and NS5B proteins of pegiviruses (used for Pin virus), the hexon protein of adenoviruses (for myna adenoviruses), the polyprotein of picornaviruses (for myna hepatovirus), the nonstructural gene of the Paroviridae (myna chaphamaparovirus) and the polyprotein for caliciviruses (myna calciviruses) determined here were aligned using the E-INS-I algorithm in MAFFT v7 [29], after which ambiguously aligned regions were removed using TrimAL employing the gappyout setting [30]. This resulted in a set of final sequence alignments that were subjected to phylogenetic analysis using the maximum likelihood (ML) method available within IQ-TREE 1.6.7 and employing the LG model of amino acid substitution with SPR branch-swapping [31]. Bootstrap resampling (1000 replications) was used to assess nodal support. The phylogenetic trees obtained were visualized using Figtree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and midpoint rooted for purposes of clarify.

2.4. Virus and host gene abundance

To compare with the extent of virus gene expression of that of host genes, the abundance of the ribosomal protein S7 (RP57) was evaluated through mapping trimmed raw reads to the RP57 gene transcript of the common starling (Sturnus vulgaris), the closest relative of the Indian myna for which an appropriate sequence is available (GenBank accession number: XM_014883722.1). RP57 has been reported to be a stable reference gene for quantitative gene expression studies in multiple avian species [32]. Putative viral reads were extracted to estimate viral abundance using RPM (reads per million) per virus genome and

### Table 1

Detailed information on the RNA-seq libraries generated in this study. The library ID, number of animals, host tissue, total trimmed reads and read abundance of each library are presented. The virus read abundance of each family and a host gene marker (RP57) are presented as Reads per Million (RPM).

| Library ID | Myna 1 L -VERT70 | Myna 2 L -VERT79 | Myna 1B -VERT80 | Myna 2B -VERT69 | Myna 1G -VERT71 | Myna 2G - VERT78 |
|------------|------------------|------------------|------------------|------------------|------------------|------------------|
| No. of animals | 8 | 8 | 8 | 8 | 8 | 8 |
| Tissue | Liver | Liver | Liver | Brain | Brain | Gut |
| Total reads | 62,477,255 | 89,923,130 | 103,780,074 | 70,380,196 | 64,848,217 | 93,735,914 |
| Flaviviridae | 44.00 | 49.82 | 0.87 | 1.25 | 0.30 | 34.22 |
| Caliciviridae | 0.19 | 0.31 | 0.019 | 0 | 0 | 0.54 |
| Picornaviridae | 0.64 | 44.99 | 0.019 | 0.057 | 0.21 | 2.44 |
| Adenoviridae | 0 | 0 | 0 | 104.39 | 0.40 | 10.42 |
| Paroviridae | 14.8 | 0 | 0 | 0 | 0 | 4.99 |
| RP57 (host) | 379.2 | 367 | 74.70 | 110.17 | 9.20 | 491.20 |

14.8 0 0 0 0 4.99
0 0 0 104.39 0.40 10.42
0.64 44.99 0.019 0.057 0.21 2.44
0 0 0 104.39 0.40 10.42
44.00 49.82 0.87 1.25 0.30 34.22
mapping against the predicted viral contigs using BBmap [33].

2.5. Screening for potential bacterial, eukaryotic and protozoan pathogens

To determine the presence (i.e. active gene expression) of any known bacterial and eukaryotic pathogen, trimmed reads were taxonomically classified using CCMetagen [34]. We specifically searched for bacteria, eukaryotes and protozoa of likely pathogenic importance, or known to infect avian species. Accordingly, the results for each microbe were presented as the number of reads per million (RPM), excluding those hits that had <0.5 RPM and less than 90% sequence similarity to a known reference sequence.

### Table 2

| Family/RPM | Myna1L-VERT70 | Myna2L-VERT79 | Myna1G-VERT71 | Myna2G-VERT78 |
|------------|---------------|---------------|---------------|---------------|
| Archaea    |               |               |               |               |
| Halorubraceae | 0            | 292.38        | 0             | 0             |
| Natrialbaceae | 0            | 85.75         | 0             | 0             |
| Bacteria    |               |               |               |               |
| Acetobacteraceae | 0        | 0             | 0             | 5.7           |
| Alcaligenaceae   | 0       | 34.36         | 0             | 0             |
| Brachyspiraceae   | 0       | 0             | 6.44          | 0             |
| Campylobacteraceae | 0     | 0             | 0             | 143.63        |
| Clostridiaceae    | 0       | 0             | 14.95         | 10.29         |
| Enterobacteriaceae | 14.86   | 0             | 18.28         | 64.4          |
| Enterococciaceae  | 0       | 0             | 0             | 16.67         |
| Erwiniaeae        | 0       | 0             | 0             | 8.54          |
| Helicobacteriaceae | 0     | 0             | 0             | 548.48        |
| Lachnospiraceae   | 0       | 0             | 5.24          | 0             |
| Mycoplasmataceae  | 15.54   | 0             | 1506.25       | 72.93         |
| Pasteurellaceae   | 0       | 0             | 0             | 0             |
| Peptostreptococciaceae | 0 | 0 | 194.59 | 0 |
| Streptococciaceae | 4.41   | 0             | 0             | 0             |
| Vibriococciaceae  | 0       | 6.53          | 0             | 105.07        |
| Fungi            |               |               |               |               |
| Glomeraceae      | 0       | 0             | 2.4           | 0             |
| Malasseziaceae   | 0.97    | 0             | 14.93         | 0.52          |
| Pleosporaceae    | 0       | 0             | 3.68          | 0             |
| Sclerotiniaceae  | 0       | 0             | 1.07          | 0             |
| Trichosphaericaceae | 0     | 0             | 0             | 5.19          |
| Eukaryota        |               |               |               |               |
| Eimeriidae       | 864.52  | 2013.98       | 1191.73       | 22186.22      |
| Geminigeriidae   | 0       | 0             | 2.62          | 0             |
| Peronosporiidae  | 2.8     | 0             | 0             | 0             |
| Plasmodiidae     | 3.14    | 89.71         | 7.73          | 4.85          |
| Salpingoecidiad  | 0       | 0             | 7.98          | 0             |
| Sarcocystidae    | 0       | 0             | 3.63          | 0             |
| Theileriidae     | 0       | 0             | 9.06          | 0             |
| Trichomonadidae  | 0       | 0             | 0             | 10.88         |

3. Results

3.1. Overview of meta-transcriptomic virome data

In total, six rRNA-depleted RNA sequencing libraries were constructed from the brain, liver and large intestine of 16 mynas, resulting in an average of 96,766,596 (ranging from 70,380,196 to 129,823,626) high-quality trimmed reads in each library (Table 1). Overall, seven novel viruses from the families Adenoviridae, Caliciviridae, Flaviviridae, Paroviridae and Picornaviridae were identified after Blast analyses to previously described viruses. The viral reads obtained were also used to estimate the abundance of each virus, with the host RPS7 gene used as reference for comparison (Table 1). Further details of each virus identified are described below. (See Table 2.)
Fig. 1. Genome characterization and phylogenetic analysis of representative pegiviruses. (A) Genome structure of Pin virus. (B) Structure of the 5’UTR region of Pin virus predicted by RNAfold. (C) Phylogenetic trees of the NS3 gene (shaded pink) and NS5B genes including the RdRp (shaded green) of selected pegiviruses. Branches are scaled according to the number of amino acid substitutions per site and bootstrap support values are shown. Pin virus is shown in red font. Trees are midpoint-rooted for clarity only. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3.2. Discovery of a novel avian pegivirus

Abundant pegivirus-related transcripts were obtained from seemingly healthy mynas. Remapping the trimmed raw reads onto the putative pegivirus-like transcript revealed a nearly completed pegivirus genome (mean read depth coverage of 143 per nucleotide position). We tentatively termed this novel virus Pin virus (Passerine INvasive myna hepatovirus) (Fig. 1). This virus was previously partially described by our group [5], and herein we provide a more complete investigation. Typical virion features of pegiviruses are a potentially infectious single-strand, positive-sense RNA, genome size ranging from 8 to 11 kb, encoding a polyprotein and possessing an IRES element. Pin virus comprises 11,053 nucleotides (nt) including a 5′ polyprotein and possessing an IRES element. Pin virus comprises 11,053 genome (mean read depth coverage of 143 per nucleotide position). We tentatively pegivirus-like transcript revealed a nearly completed pegivirus.

3.3. Myna calicivirus and a novel myna hepatovirus

Additional myna-associated RNA viruses were identified from the families Picorniviridae and Caliciviridae. Specifically, an hepato-like virus (Picornaviridae) transcript was identified in the liver and gut RNA-seq libraries. The length of the nearly complete virus genome was 7928 nt (mean read depth of 149 reads/position), encoding a polyprotein of 2208 amino acid residues. A phylogenetic analysis of the polyprotein revealed that this novel picornavirus was most closely related to Garigal virus (NCBI accession number: MT457854.1), previously identified from a rainbow lorikeet hepatovirus in Australia [19], with which it shared 70% polyprotein amino acid similarity. Notably, Garigal virus was found in both diseased and non-diseased lorikeets (Fig. 2).

Two partial calicivirus transcripts, a 945 nt and a 2444 nt fragment of the partial polyprotein containing RdRp, were obtained from the gut library (Myna-2G VERT78) (Table 1). Phylogenetic analysis of the polyprotein demonstrated that the two caliciviruses clustered with other avian caliciviruses, forming a distinct avian clade. BLASTx searches indicated that both fragments were most closely related to a sequence denoted as Caliciviridae sp. (NCBI accession number: QKN88791.1), identified from bird fecal metagenomic samples from China and exhibiting 52.96% and 58.49% amino acid sequence identity, respectively (Fig. 3).

3.4. Novel DNA viruses identified in mynas

Adenoviruses have been detected in a variety of vertebrate hosts from fish, reptiles, marsupials as well as avian species worldwide. Most avian-related adenoviruses belong to three genera: Aviadenovirus, Siaadenovirus and Atadenovirus [35]. We identified several abundant transcripts associated with aviadenviruses and siadenviruses, which were reassembled into two partial genomes of adenoviruses and denoted here as myna aviadenovirus and myna siadenvirus. Hexon gene sequences were then used to infer phylogenetic relationships among adenoviruses. Accordingly, myna aviadenovirus exhibited 78.26% amino acid similarity with its closest documented relative, Southern Patas tarsus leucophthalmus aviadenovirus (NCBI accession number: MN153802.1), identified from the feces of white-eye parakeets in Brazil [36]. Similarly, myna siadenvirus, shared 78.69% amino acid similarity with its closest relative - Great tit adenovirus 1 (NCBI accession number: NC_043405.1) isolated from a diseased great tit (Parus major) in Hungary [37].

![Fig. 2. Phylogenetic analysis of myna hepatovirus. Phylogenetic tree of the polyprotein, containing the RdRp region, of relevant picornaviruses focusing on the novel myna hepatovirus identified here (bold red font). The tree was midpoint rooted, corresponding to the division between mammalian and avian viruses. The blue box indicates members of the genus Hepatovirus. Bootstrap values >70% are shown for key nodes. The scale bar indicates the number of amino acid substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](attachment://image.png)
To compare our myna associated adenoviruses to those identified previously in birds from New South Wales [6], nucleotide sequences of the DNA polymerase gene (~232 nt to accord with the data provided in [6]) were incorporated into a broader phylogenetic analysis. This revealed that myna avianadenovirus clustered with Aviadenovirus spp. isolates WHC1055 and WHC1073 (NCBI accession number: MN238650.1 and MN238651.1), identified from the feces of the Eastern yellow robin (Eopsaltria australis) and Eastern shrike-tit (Falcunculus frontatus), although exhibiting only ~38% nucleotide similarity. Similarly, the myna siadenovirus shared 68% nt identity with the Siadenovirus sp. isolate WHC1110 (MN238652.1) detected in the Silverseve (Zosterops lateralis). Arguably of most note was that the myna siadenovirus only shared 65% similarity with a siadenovirus previously identified in the kidney of an invasive myna in Australia (accession MN238647.1), suggesting an understudied diversity of myna adenoviruses (Fig. 4B).

Finally, a myna chaphamaparvovirus (mchPV) was detected from an intestinal RNA-seq library (VERT71). In the case of eukaryotes, sequence reads for the Plasmodiidae, ranging from 864 to 22,186 (RPM) across all liver and gut libraries. The most abundant eukaryote family identified were the Eimeriidae, a family of protozoans of the order Coccidia that includes the Eimeria and Isospora genera that are of medical and veterinary importance. The transcripts associated with Eimeriidae were retrieved and reassembled into one complete mitochondrial genome of and chestnut teal chaphamaparvovirus 1. (Fig. 5). The amino acid sequences of mchPV shared only 36.99% pairwise identity in NS gene (NCBI accession: AXL64655.1) and 31.14% in VP gene (NCBI accession: AXL64656.1), respectively, to chicken chapparvovirus 2. (See Fig. 6.)

3.5. Microbial profiling and the identification of Isospora spp.

Both gut libraries showed a greater diversity in the number of bacterial families compared to the liver. The most abundant bacterial family of interest in the gut was the Mycoplasmataceae (RPM 1506.25). However, the patterns of bacterial composition of the two gut libraries were different, which may be related to animal diet, sample location and/or individual variation. The most abundant fungi in the data set were members of the Malasseziales, observed at low abundance in the gut library (myna1G- VERT71). In the case of eukaryotes, sequence reads for the Plasmodiidae were found in all libraries but at relatively low abundance, ranging from 3 to 90 RPM (Fig. S2). This result is unsurprising given previous descriptions of malaria in Indian mynas in Australia [4].

Notably, the dominant eukaryote family identified were the Eimeriidae, ranging from 864 to 22,186 (RPM) across all liver and gut libraries. The Eimeriidae is a family of protozoans of the order Coccidia that includes the Eimeria and Isospora genera that are of medical and veterinary importance. The transcripts associated with Eimeriidae were retrieved and reassembled into one complete mitochondrial genome of...
Fig. 4. Phylogenetic relationships of the myna adenoviruses. (A) Amino acid phylogenetic tree of the hexon protein of myna siadenovirus and myna aviadenovirus (bold red font). (B) Phylogenetic tree of DNA polymerase (~232 nt) of the myna aviadenovirus and myna siadenovirus (bold red font). The yellow box shading indicates the avian siadenoviruses while the purple box denotes the avian aviadenoviruses. The scale bar represents the number of substitutions per site and bootstrap values are shown. Both trees are mid-point rooted for clarity only. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Isopora, termed *Isopora greineri* strain/vert71len6461 to reflect its closest match to known taxa. The genome of this *Isopora* strain (6461 nt) encoded typical features of mitochondrial protein coding genes, including the cyb, COX as well as LSU and SSU regions. The full genome showed 99.2% nucleotide similarity to *Isopora greineri* voucher MAH-2013a_MTZ1 (accession number: KR108298.1) [38]. A near complete fragment of *Isopora superbusi* strain/vert78len2672 (5770 bp) was also identified, sharing 98.78% nt similarity with strain MTZ22 (accession number: KT203396.1) [38]. The cytochrome c oxidase subunit 1 (COX1) gene regions of these coccidian protozoa were then utilized for phylogenetic analysis. Notably, both the *Isopora* sequences identified in our myna samples were closely related to *Isopora greineri* (98.96% nt identity) and *Isopora superbi* (99.38% nt identity) isolated from superb glossy starlings (*Lampropterus superbus*) from Canada [38], clustering in the same clade as *Isopora sp*. identified in other Passeriformes.

4. Discussion

The Indian myna bird is a highly invasive avian species in urban Australia that frequently interacts with native species, peri-urban and rural agricultural species, and humans. Herein, we present the first metagenomic survey of the virome of highly invasive Indian myna birds
Accordingly, future work should increase both the sample size and the number of sites, addressing whether there are host genetic or environmental barriers to successful cross-species virus transmission. However, the apparent lack of virus transmission between invasive and native species may simply reflect a lack of sampling. Indeed, all the viruses identified here were genetically highly distinct. For example, while the myna hepatovirus newly identified here clustered with its closest relative, Garigal virus, a lorikeet hepatovirus previously identified in Australia [19], it only shared ~45% amino acid similarity in the polyprotein sequence, suggesting a long period of independent evolution. However, the apparent lack of virus transmission from invasive to native species may simply reflect a lack of sampling. Accordingly, future work should increase both the sample size and the number of sites, addressing whether there are host genetic or environmental barriers to successful cross-species virus transmission.

As in many recent avian virome studies, most of the newly identified myna-associated viruses were from families Picornaviridae, Parvoviridae, Circoviridae, and Caliciviridae [40]. Generally, all the closest relatives of the viruses discovered in this study were avian viruses, indicative of a relatively long evolutionary history in birds. The novel Pin virus described here is of particular interest as this represents only the second pegivirus from an avian species. Goose pegiviruses isolated from China showed lymphotropic pathogenicity and a high rate of co-infection with paroviruses, circoviruses and astroviruses, with a co-infection frequency of 24.5% between goose pegivirus and goose parovirus [41]. Of note, Pin virus exhibited higher abundance in liver libraries in comparison to other organs, suggesting its potential hepatotropism or lymphotropism in accordance with those pegiviruses found in pigs, horses and cetaceans [10,42,43]. Coincidently, we identified a novel myna chaphamaparvovirus (Paroviridae) in the same sequencing library as Pin pegivirus. It is unclear whether this coinfection is of clinical or epidemiological importance, or whether Pin virus alters host fitness in mynas and other birds.

Many studies have revealed a high diversity of adenoviruses in a range of Australian birds [6,36,44,45]. In addition, recent studies have shown that adenoviruses frequently jump species boundaries to emerge in new hosts [46], although adenoenoviruses identified from Psittaciformes tend to cluster together in phylogenetic trees [35]. Interestingly, the partial DNA polymerase sequence previously detected in an Indian myna [6] (NCBI accession: MN238647) was identical to those found in native Australian species (honeyeater), yet only shared 65% nucleotide similarity with the myna siadenovirus identified in our study. Although the polymerase region used for phylogenetic analysis is short (232 nt), this result suggests that this invasive species might be susceptible to infection by enzootic avian siadenoviruses, and that additional adeno-viruses are likely to be present in this species. Importantly, however, we found no evidence of clustering of myna-associated viruses with those previously sampled from native bird species.

In addition to viruses, our meta-transcriptomic analysis of mynas revealed multiple bacteria, fungi and protozoa. Of particular interest was the identification of two mitochondrial genomes of isosporan coccidial parasites. Over 90% of the described coccidia infecting wild birds belong to the genus Isospora, either sub-clinically or in association with anorexia and enteritis [47]. It has been suggested that Isospora species
are highly host specific at the genus level [48]. However, their phylogenetic position in our study suggests possible isosporan transmission between bird species within the same family, since the myna and superb glossy starling are both members of Stornidae.

More broadly, this study demonstrates the value of performing transcriptome sequencing to simultaneously detect an array of viruses, bacteria, and eukaryotes (fungi and protozoa). Notably, economically important zoonotic pathogens such as Salmonella typhimurium DT160 [49], Chlamydia psittaci [50] and avian pathogenic Escherichia coli [51,52], which have been previously reported to cause zoonoses and mortality outbreaks in wild and domestic avian species, were not detected in our genomic data. Avian malaria parasites (Plasmodium spp. and Haemoproteus spp.) have previously been demonstrated to be relatively abundant in mynas [5]. Interestingly, we detected abundant Plasmodium reads in the liver of mynas, although no Haemoproteus reads were obtained. Haemoproteus spp. were mostly recorded in the primary range of the myna and not at their secondary expansion sites [4], which is compatible with their absence from Sydney, Australia.

The surveillance of invasive species in urban areas is central to the identification of known and novel microbes of significant concern to the health of wildlife, domestic animals and humans, and providing important evolutionary context. Given the high density and invasiveness of wildlife, domestic animals and humans, and providing improvements in performance and usability, Mol. Biol. Evol. 30 (2013) 772-786.

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Data availability statement
The raw sequence reads are available at BioProject PRJNA756632. Consensus sequences of the new viruses identified in this study are available on GenBank under accession numbers OK334621 to OK334628.

Declaration of competing interest
The authors declare no conflict of interest.

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