**Supplementary Information**

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**COI DNA Barcoding methodology to determine tissue provenance**

Residual genomic DNA present in the RNA extraction was used to amplify the *COI* barcoding region gene to support the species morphological identification. The COI barcoding region was amplified using the primers LCOI490 and HCO2198 (Folmer *et al*., 1994). PCR reactions were performed in a total volume of 50 µL (Hernandez-Triana *et al*., 2017) using 2 µL of genomic DNA, 1 X $NH_4$ buffer, 2 pmol/µL dNTPs, 1.5 mM $MgCl_2$, 10 pmol/µL of each primer, 0.6U *Taq* DNA polymerase (Bioline, UK) and 20 mg/mL bovine serum albumin. The thermal profile consisted of 1-min initial cycle at 94°C followed by a pre-amplification 5 cycles of 94°C for 1 min, 45°C for 1.5 min, 72°C for 1.5 min, and an amplification step of 35 cycles of 94°C for 1 min, 57°C for 1.5min, 72°C for 1.5 min with a final extension of 72°C for 5 min. PCR products were separated by electrophoresis in 1.5% agarose gel and samples showing the expected band size were purified using the QIAquick PCR purification kit (Qiagen, UK) and sequenced in both directions using the ABI PRISM® BigDye® Terminator sequencing kit (Applied Biosystems, UK) following the manufacturer´s instructions. All sequences were assigned to a species when agreement was ≥96% with sequences of named species in GenBank. Tissue samples 1-5, described as blackbird and tissue sample 6 described as a house sparrow were confirmed using this *COI* method.

**References**:

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.*Molecular Marine Biology and Biotechnology*. **3**: 294–299.

Hernández-Triana, L.M., Brugman, V.A., Prosser, S.W.J., Weland, C., Nikilova, N., Thorne, L., de Marco, M.M.F., Fooks, A.R. & Johnson, N. (2017) Molecular approaches for blood meal analysis and species identification of mosquitoes (Insecta: Diptera: Culicidae) in rural locations in southern England, United Kingdom. *Zootaxa*, **4250**:067-076.

**Negative immunohistochemistry slides for a RT-PCR negative brain and kidney sections from a blackbird and house sparrow**

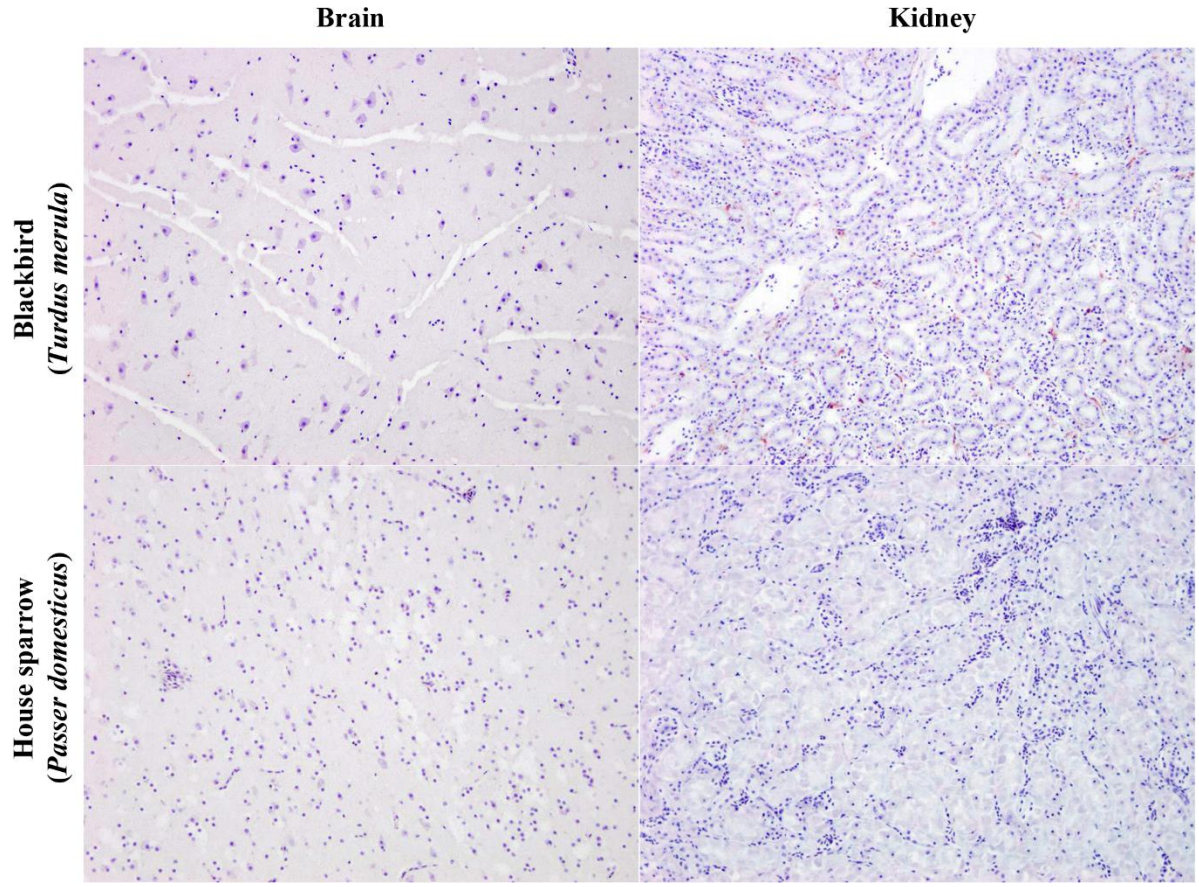


Figure 1. Immunohistochemical labelling of formalin-fixed paraffin-embedded tissue sections obtained from USUV RT-PCR negative blackbird (*Turdus merula*) and house sparrow (*Passer domesticus*). Images taken at 200x magnification.

**Phylogenetic analysis and Genbank sequence provenance**

Table 1. Accession numbers used for alignment against Greater London 2020 sequence (accession number MW001216) and subsequent phylogenetic analysis.

| Accession number | Length (bp) | Representative lineage |
|---|---|---|
| KC754958 | 10745 | Africa 1 |
| AY453412 | 11064 | Africa 2 |
| MN122238 | 10932 | Africa 3.1 |
| MN122245 | 10932 | Africa 3.2 |
| MN122246 | 10932 | Africa 3.2 |
| MN122249 | 10932 | Africa 3.2 |
| MN122254 | 10932 | Africa 3.2 |
| MN122237 | 10932 | Africa 3.3 |
| MN122239 | 10932 | Africa 3.3 |
| MN122242 | 10932 | Africa 3.3 |
| MN122252 | 10932 | Africa 3.3 |
| MN122256 | 10932 | Africa 3.3 |
| MN122196 | 10932 | Europe 3 |
| MN122213 | 10932 | Europe 3 |
| MN122215 | 10932 | Europe 3 |
| MN122229 | 10932 | Europe 3 |
| MN122235 | 10932 | Europe 3 |

**Consensus sequence read coverage depths aligned to a *de novo* assembled contig that matched an Africa 3.2 lineage of Usutu virus.**

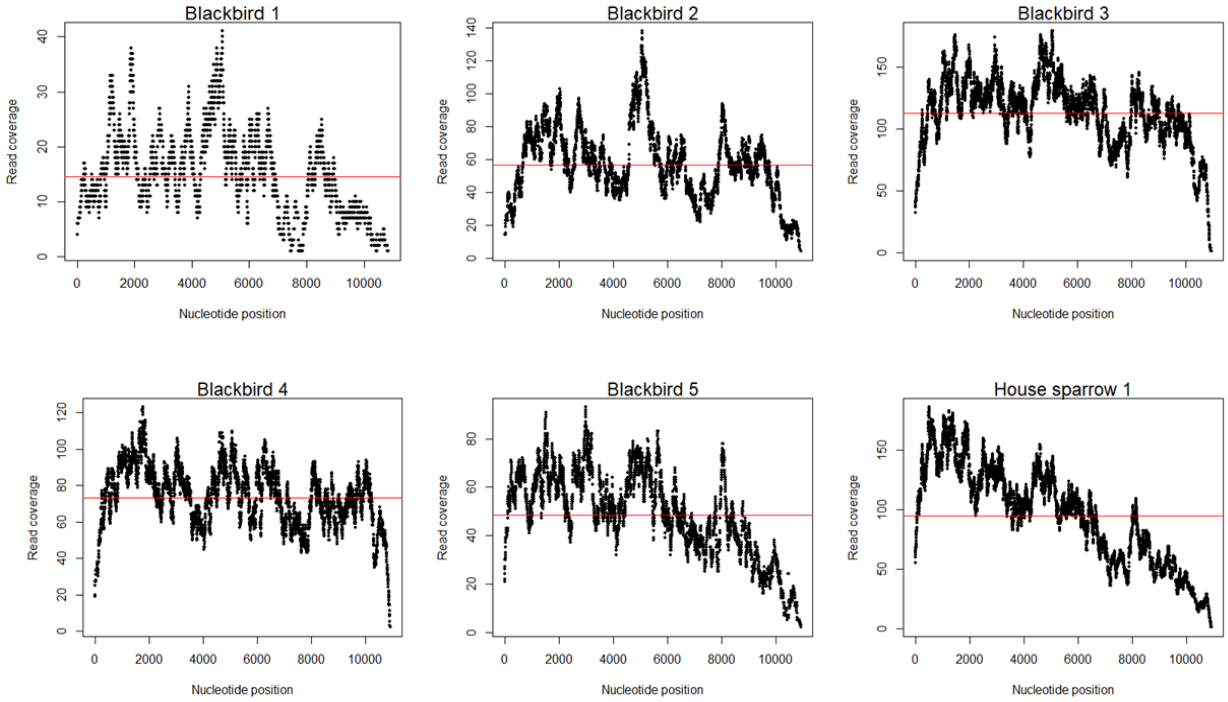


Figure 2. Read coverage plots for consensus sequences, aligned to a *de novo* contiguous sequence (10,922 bp), for five blackbird and one house sparrow RNA samples. The average coverage depth is marked with a red line.

**Additional references for NGS analysis /phylogenetic packages and diagnostic PCRs**


**SPAdes**= Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikelenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: A new genome assembly algorithm and its applications to single cell sequencing. *Journal of Computational Biology*. **19**: 455-477.

**BWA** = Li, H., Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. **25**: 1754-1760.

**SAMtools** = Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. (2009) The sequence alignment/Map format and SAMtools. *Bioinformatics*. **25**: 2078-2079.

**Tablet** = Milne, I., Stephen, G., Bayer, M., Cock, P.J., Pritchard, L., Cardle, L., Shaw, P.D., Marshall, D. (2013) Using Tablet for visual exploration of second-generation sequencing data. *Briefings in Bioinformatics*. **14**:193-202.

**Mafft** = K., Katoh, Misawa, K., Kuma, K., Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acid Research*. **30**: 3059-3066.

**BEAST** = Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A. (2018) Bayesian phylogenetic and phylogenomic data integration using BEAST 1.10. *Virus Evolution*. **4**: vey016

**Tracer** = Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*. **67**: 901-904.

**FigTree** = Rambaut, A. (2014) Figtree v1.4.2 A graphical viewer of phylogenetic trees. Available from (http://tree.bio.ed.ac.uk/software/figtree/)