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Additional records of the tropical nest fly, *Passeromyia heterochaeta* Villeneuve, 1915 (Diptera: Muscidae) from western Iran supported by DNA barcoding

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Abstract

Passeromyia is a genus of flies associated with birds' nests. Adults of *Passeromyia heterochaeta* were found within the houses near to domestic pigeon nests in Lorestan Province, western Iran. Flies were identified at the genus level by analysis of *COI* sequences of a representative specimen. Then, the specimens were identified according to morphological characteristics as *P. heterochaeta* and the sequence was submitted to the GenBank under this name. DNA barcoding approach can be used for approximate identification before using morphological keys.

Key words: Bird myiasis, *COI* barcoding, phylogenetic tree, Lorestan Province

Introduction

Muscid flies of the genera *Mydaea* Robineau-Desvoidy, *Passeromyia* Rodhain and Villeneuve, *Phiilornis* Meinert, and *Protocalliphora* Hough are called tropical nest flies. These species usually parasitize the young of cavity-nesting birds by sucking nestlings' blood externally, or by burrowing under the skin or into the body cavity to feed on blood and tissues (Moon, 2019). The genus *Passeromyia* encompasses a small group of species found in the tropical areas of the old world, from tropical-South Africa to East Asia, and eastwards to Australia and the Indo-West Pacific (Couri and Carvalho, 2003). Currently, five species of the genus *Passeromyia* are recognized: *Passeromyia heterochaeta* Villeneuve, *P. indecora* (Walker), *P. steini* Pont, *P. longicornis* Macquart, and *P. veitchi* Bezzi (Couri and Carvalho, 2003). *Passeromyia heterochaeta* and *P. longicornis* are species with considerable synonymies by various authors (Pont, 1974).

All *Passeromyia* species are associated with birds' nests, and the larvae are scavengers in the nest debris (*P. steini*), external bloodsuckers of nestlings (*P. heterochaeta*), or subcutaneous parasites of nestlings (*P. indecora*) (Kutty et al., 2014). In the last case, if the host dies, the subcutaneous larvae can feed on the carcass until ready to pupate (Couri and Carvalho, 2003).

Endangered species of birds such as the Echo parakeet, *Psittacula eques* (Boddaert), and Forty-spotted pardalote, *Pardalotus quadragintus* Gould can be severely affected by *Passeromyia* larvae as high infestations can lead to death of nestlings (Jones, 2004; Little, 2008; Edworthy, 2016). Parasite prevalence and nestling mortality of Forty-spotted pardalote,

P. quadragintus, when parasitized by *Passeromyia longicornis* was 65% to 85% (Edworthy, 2016).

Passeromyia heterochaeta was first described by Villeneuve (1915) as *Muscina heterochaeta* from Africa. But, Coutant (1915) reported a fly larva in the nest of the Northern Grey-headed sparrow, *Passer griseus* (Vieillot), in Congo that was formerly figured as an unknown bloodsucking larvae by Rodhain (1914). He found that these larvae contained avian blood. They were reared but the adult fly had not been determined. Parasitic larvae of *P. heterochaeta* were also found in the nests of various passerine birds species such as the house sparrow (*Passer domesticus*), the Cape canary (*Serinus canicollis*) and the Common fiscal (*Lanius collaris*) (Hicks, 1959). The species was recorded in Iran for the first time from Qeshm island in the Persian Gulf, southern Iran (Grzywacz et al., 2014; Khoobdel et al., 2015). After an outbreak of a muscid fly in a colony of the nests of the Rock Dove, *Columba livia* Gmelin, constructed in human buildings in western Iran, the author aimed to identify it to the species level, both morphologically and molecularly.

Material and Methods

Sample collection, DNA extraction and PCR

This study was conducted during two successive summers in 2018–2019. The adult flies were captured by a long glass tube within residential houses located in Khorramabad city of western Iran (33°29'18 N, 48°24'49 E and 1382 m a.s.l.). Insect specimens were identified to species level under a stereomicroscope (Wild-Heerbrugg M8Model) according to the morphological keys of Bezzi (1922), Zumpt (1965), and Pont (1974). To identify and prepare the flies, photos of body parts (head, thorax, wing, leg, antenna, and abdomen) were captured using a digital camera (Nikon® Coolpix S7000). Voucher specimens are retained in the Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences.

Genomic DNA of a representative specimen was extracted using CTAB according to Doyle and Doyle (1987) with minor modifications. A 524 bp fragment of the cytochrome oxidase subunit I (*COI*) was amplified by polymerase chain reaction (PCR) using the primers designed by Simon et al. (1994) including C1-J-1718: 3′- GGA GGA TTT GGA AAT TGA TTA G -5′ and C1-N-2191: 3′- CCC GGT AAA ATT AAA ATA TAA ACT TC -5′ with minor modification in C1-J-1718 as follows: deletion of –TTCC– from the 5′ end. PCR reactions were carried out in a thermocycler (Corbett®, Australia) based on a touchdown temperature profile: 3 minutes at 94 °C, 11x [45 s at 94 °C, 50 s at 60 °C, 60 s at 72 °C], followed by 24x [45 s at 94 °C, 50 s at 50 °C, 60 s at 72 °C], 3 minutes at 72 °C). PCR for each 25 µl final volume reaction was performed using 12.5 µl RedMaster PCR 2X (Sinaclon®, Iran), 1 µl of each primer (10 pM), 4 µl gDNA template (100 ng/µl), and 6.5 µl ddH_2O. The PCR products were visualized with 1% agarose gel electrophoresis, and the desired bands were purified using the GF-1 Gel DNA Recovery Kit (Vivantis®, Malaysia). Finally, the purified PCR products were submitted to a third-party service provider for sequencing (Faza-Biotech® Inc., Iran).

Phylogenetic analysis

The DNA sequence was manually checked using FinchTV® software (www.geospiza.com) to correct any sources of error or ambiguities if present. Homologies with the available sequence data in GenBank were checked using BLAST analysis. Finally, sequences were submitted to GenBank under accession number (MN197540). Then, the sequences were aligned using

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SeaView4 software (Gouy et al., 2010). The genetic distances among and between sequences were calculated using Maximum Composite Likelihood (MCL) modeled in MEGA7 (Kumar et al., 2016). To construct the phylogenetic tree, a 362 bp alignment sheet was analysed using BEAST® (Ver. 2.6.0) (Bouckaert et al., 2014) based on the Bayesian Inference (BI) method.

We selected an appropriate substitution model using the FINDMODEL program (http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html) (Posada and Crandall, 1998), which identified Jukes-Cantor (JC69) as the appropriate tree model. BI employs Markov Chain Monte Carlo (MCMC) algorithms and infers a most credible tree given the posterior probabilities of alternative tree topologies. For this purpose 10 taxa, including a sequence of the present study (MN197540), as well as the comparable data sequences of the target species (BOLD system accession ACR7907, GenBank unpublished accession MN410989), genus (KJ510635, KY937944-5) and outgroup taxa (KF652213, KU932127, KU932138, KU932145, KY001858), were used. Nine sequences of *COI* of related muscid genera were selected according to the similarity revealed by the BLAST algorithm. The outgroups were chosen according to Smith (1994) and Wenzel (2002). They suggested that outgroups could be selected from sister groups as well as successively more distant lineages. Thus, the genera *Azelia*, *Haematobia*, *Hydrotaea*, *Potamia* and *Synthesiomyia* were examined as outgroups. The tree was summarized and visualized using TreeAnnotator and FigTree (Ver. 1.1.4.), respectively.

Results

Fly collection, identification and BLAST analysis

In total, thirty-three adult specimens of flies were collected indoors during July and August (rarely September) 2018–2019 at houses located in the countryside near Khorramabad City, Lorestan Province. BLAST analysis of the nucleotide sequence from one of the individuals showed 98%, 89% and 90% sequence identity to *COI* sequences of *Passeromyia heterochaeta* (BOLD accession ACR7907 submitted from India), *P. longicornis* and *P. indecora*, respectively. Then, the specimens were recognized as *P. heterochaeta* based on described morphological characters, including post-alar declivity, haired eye, shifting tessellated pattern of the abdomen, epaulet, squamae, parafrontalia and parafacialia, scutellum, 3rd antennal segment, wing venation, ciliated hind tibiae and bristles of the femora (Fig. 1). These traits differ in the other species of the genus *Passeromyia*. Iranian distribution records of *Passeromyia heterochaeta* were updated as a map (Fig. 2).

Phylogenetic analysis

A phylogenetic tree was constructed based on partial *COI* sequences. The phylogeny of genus *Passeromyia* is rooted with a clade of selected Muscidae genera (*Haematobia*, *Azelia*, *Hydrotaea*, *Potamia*, *Synthesiomyia*) as outgroups (Fig. 3). The *COI* phylogenetic tree shows a clade of *Passeromyia* with five members, including the sequence of this study (MN197540), *P. heterochaeta* (BOLD accession ACR7907, GenBank unpublished accession MN410989) from India, *P. longicornis* (KY937944) and two *P. indecora* (KJ510635-KY937945) from Australia. The genetic distance between different species of the *Passeromyia* clade (*P. longicornis*, *P. indecora*, and *P. heterochaeta*) is 6% – 13%.

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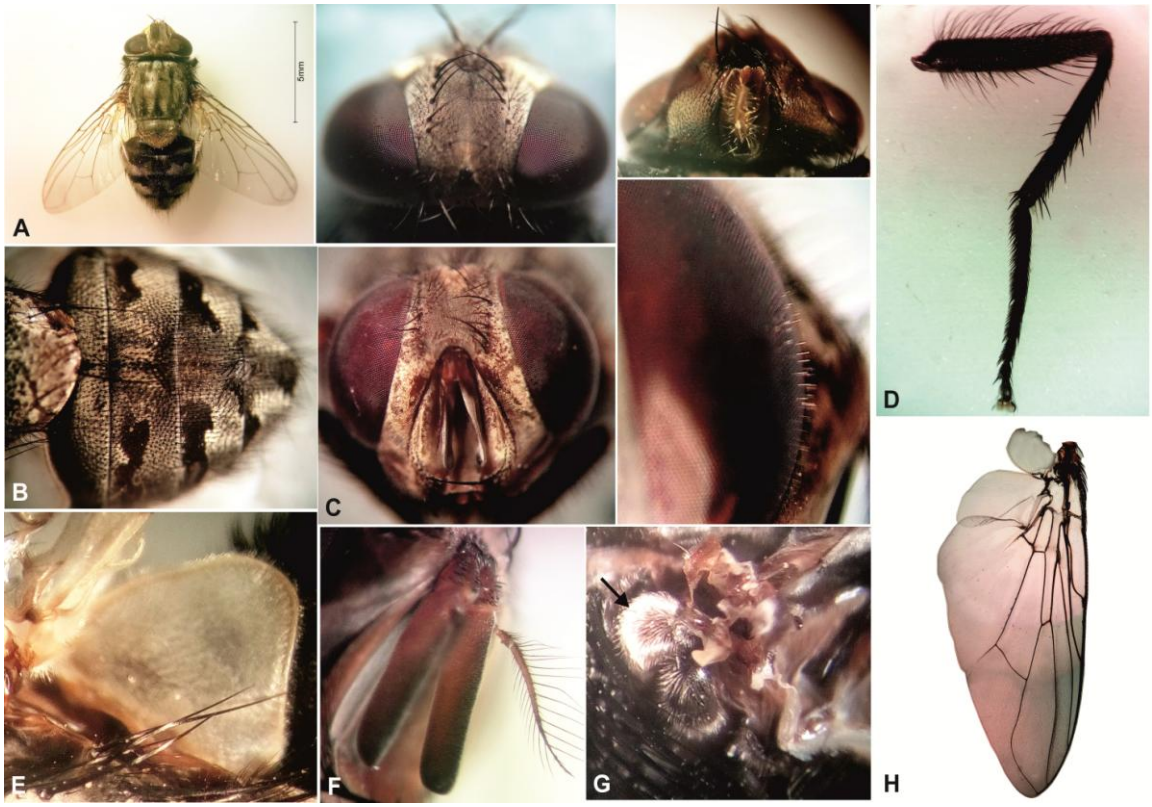

Figure 1: Diagnostic morphological characteristics of adult *Passeromyia heterochaeta*; General body shape from above (A), shifting tessellated pattern of abdomen and scutellum (B), head: clockwise including anterior, and dorsal view (parafrontalia), ventral view (parafacialia) with lighter pruinose, varying from brown through golden to silvery white, and haired compound eye, respectively (C), ciliated hind tibiae and bristled femora (D), squamae (E), third antennal segment not reaching the border of the mouth (F), epaulet orange (arrow) G), and wing venation pattern (H).

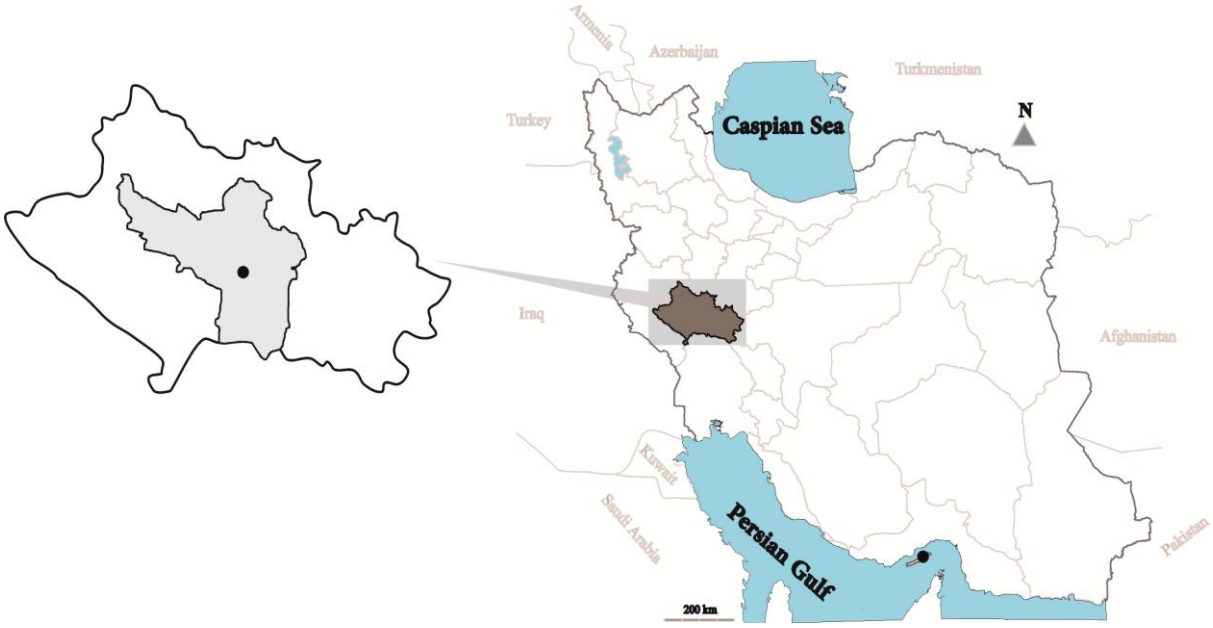

Figure 2: Current distribution map of *Passeromyia heterochaeta* in Iran (solid circles) including records of this study in Khorramabad City, Lorestan Province, Western Iran as well as, Qeshm Island in the Persian Gulf, Southern Iran.

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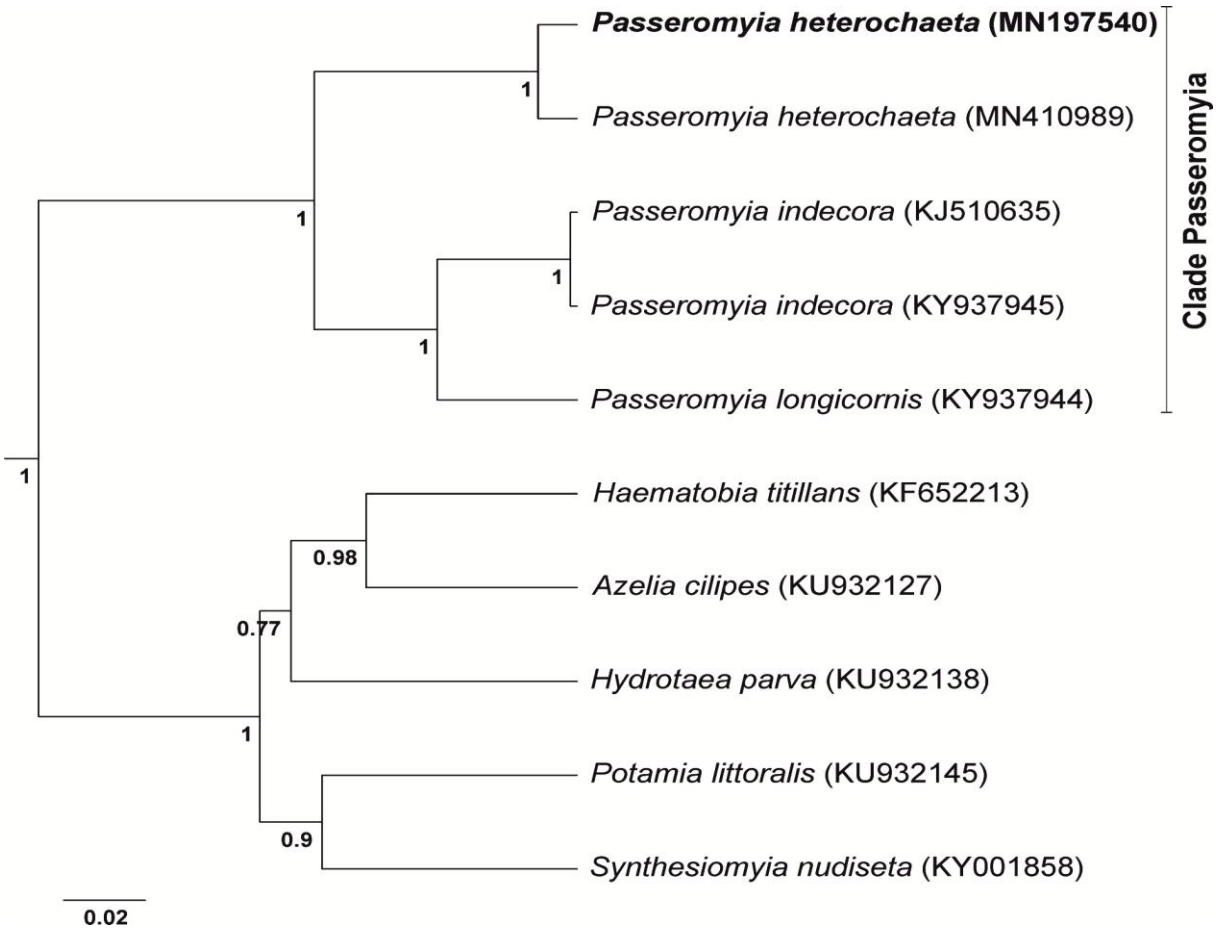

Figure 3: Phylogenetic relationships among selected muscid fly taxa and species of the genus *Passeromyia* derived from the Bayesian inference (BI) generated based on analysis of partial cytochrome oxidase subunit I (*COI*); numbers below each node show posterior probability value greater than 0.98 (10 million reiterations). Taxon labels give the species name followed by GenBank accession numbers in parentheses; the taxon sequenced in the present study is highlighted in bold. Branch lengths are proportional to the evolutionary distances.

Discussion

The adults of *Passeromyia heterochaeta* were found within the houses near to domestic pigeon nests in Lorestan Province, western Iran. First, flies were identified at the genus level by analysis of cytochrome oxidase subunit 1. Then, the specimens were identified as *P. heterochaeta* according to the morphological characteristics. In the past a parasitic larva of *P. heterochaeta* was found on one nestling red bishop, *Euplectes orix* in South Africa (Lindholm et al., 1998). Tropical nest fly, *P. heterochaeta* larvae feed on nestling Echo Parakeets (*Psittacula eques*) and can be major source of mortality (Jones, 2004). *Passeromyia indecora* causes hematophagous myiasis in captive-bred domestic pigeon (*Columba livia domestica*) squabs (Nelson and Grzywacz, 2017). *Passeromyia longicornis* infests pardalotes (family Pardalotidae) in south-eastern Tasmania (Edworthy, 2016).

BLAST analysis showed 98%, 89% and 90% sequence identity of our sequence to *COI* sequences of *Passeromyia heterochaeta* and species referring to the other members of the genus *Passeromyia*. The use of a DNA barcoding approach provides a helpful, and alternative, diagnostic tool to confirm a morphological-based identification if the reference database includes the species. Alternatively, it can be used for approximate identification

before using morphological keys. Our phylogenetic analysis supported the monophyly of genus *Passeromyia* (*P. longicornis*, *P. indecora* and *P. heterochaeta*). Based on previous cladistic analysis, *Passeromyia* formed a monophyletic group with closely related genera *Synthesiomyia*, *Calliphoroides*, and *Reinwardtia* (Couri and Carvalho, 2003).

In our analysis, the genus *Passeromyia* was relatively isolated from other genera including blood sucking *Haematobia*. The 13% genetic distance between *P. heterochaeta* and *P. longicornis* suggests a long independent evolutionary history. This study is the first record of the occurrence of *P. heterochaeta* in mainland Iran and provides only the second *COI* sequence for the species.

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References

Bezzi, M. (1922). On the dipterous genera *Passeromyia* and *Ornithomusca*, with notes and bibliography on the non-pupiparous Myiodaria parasitic on birds. *Parasitology*, 14 (1): 29–46. https://doi.org/10.1017/S0031182000009938

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A. and Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLOS Computational Biology*, 10 (4): e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Couri, M. S. and Carvalho, C. J. B. (2003). Systematic relations among *Philornis* Meinert, *Passeromyia* Rodhain & Villeneuve and allied genera (Diptera, Muscidae). *Brazilian Journal of Biology*, 63 (2): 223–232. http://dx.doi.org/10.1590/S1519-69842003000200007

Coutant, A. F. (1915). The habits, life history, and structure of a blood-sucking muscid larva (*Protocalliphora azurea*). *The Journal of Parasitology*, 1 (3): 135–150. http://dx.doi.org/10.2307/3271117

Doyle, J. J. and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19 (1): 11–15.

Edworthy, A. B. (2016). Avian hosts, prevalence and larval life history of the ectoparasitic fly *Passeromyia longicornis* (Diptera: Muscidae) in south-eastern Tasmania. *Australian Journal of Zoology*, 64 (2): 100–106. https://doi.org/10.1071/ZO15060

Gouy, M., Guindon, S. and Gascuel, O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27 (2): 221–224. https://doi.org/ 10.1093/molbev/msp259

Grzywacz, A., Khoobdel, M. and Akbarzadeh, K. (2014). First Palaearctic record of the bird parasite *Passeromyia heterochaeta* (Diptera: Muscidae) from the Iranian Persian Gulf Islands. *Journal of Arthropod-Borne Diseases*, 8 (2): 224–227.

Hicks, E. A. (1959). *Check-list and bibliography on the occurrence of insects in birds' nests*. Ames, Iowa State College Press, Iowa, USA. 681 pp.

Jones, C. G. (2004). Conservation management of endangered birds, *In*: Sutherland, W. J., Newton, I. and Green, R. E. (Eds.), *Bird ecology and conservation: A handbook of*

[DOR: 20.1001.1.2676685.2019.1.1.5.4]

[DOI: 10.29252/JAD.2019.1.1.5]

techniques. Techniques in Ecology and Conservation Series, Oxford University Press, Oxford, UK. pp. 269–301.

Khoobdel, M., Akbarzadeh, K. and Rafinejad, J. (2015). Fauna and relative frequency of synanthropic flies in the biggest Persian Gulf Island, Qeshm, Iran. *Journal of Coastal Life Medicine*, 3 (9): 733–736. http://dx.doi.org/10.12980/jclm.3.2015j5-116

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33 (7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Kutty, S. N., Pont, A. C., Meier, R. and Pape, T. (2014). Complete tribal sampling reveals basal split in Muscidae (Diptera), confirms saprophagy as ancestral feeding mode, and reveals an evolutionary correlation between instar numbers and carnivory. *Molecular Phylogenetics and Evolution*, 78: 349–364. https://doi.org/10.1016/j.ympev.2014.05.027

Lindholm, A. K., Venter, G. J. and Ueckermann, E. A. (1998). Persistence of passerine ectoparasites on the diederik cuckoo *Chrysococcyx caprius*. *Journal of Zoology*, 244 (1): 145–153. https://doi.org/10.1111/j.1469-7998.1998.tb00016.x

Little, S. E. (2008). Myiasis in wild birds, *In*: Atkinson, C. T., Thomas, N. J. and Hunter, D. B. (Eds.), *Parasitic diseases of wild birds*. Wiley-Blackwell, Ames, Iowa, USA. pp. 546–556.

Moon, R. D. (2019). Muscid flies (Muscidae), *In*: Mullen, G. and Durden, L. (Eds.), *Medical and veterinary entomology*. Academic Press, London, UK. pp. 345–368.

Nelson, L. and Grzywacz, A. (2017). *Passeromyia indecora* causes hematophagous myiasis in captive-bred domestic pigeon (*Columba livia domestica*) squabs. *Memoirs of the Queensland Museum*, 60: 172–173. https://dx.doi.org/10.17082/j.2204-1478.60.2017.2017-03

Pont, A. C. (1974). A revision of the genus *Passeromyia* Rodhain & Villeneuve (Diptera: Muscidae). *Bulletin of the British Museum (Natural History)*, 30 (7): 339–372. https://doi.org/10.5962/bhl.part.24943

Posada, D. and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14 (9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rodhain, J. (1914). Sur une larve de Muscinae vivant dans le nid de *Passer griseus*, au Congo. *Revue Zoologique Africaine*, 3: 213–217. [in French]

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87 (6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Smith, A. B. (1994). Rooting molecular trees: problems and strategies. *Biological Journal of the Linnean Society*, 51 (3): 279–292. https://doi.org/10.1111/j.1095-8312.1994.tb00962.x

Villeneuve, J. (1915). Diptères nouveaux d'Afrique. *Bulletin de la Société entomologique de France*, 20 (14): 225–227. [in French]

Wenzel, J. W. (2002). Phylogenetic analysis: the basic method, *In:* DeSalle, R., Giribet, G. and Wheeler, W. (Eds.), *Techniques in molecular systematics and evolution*. Springer, Basel, Switzerland. pp. 4–30.

Zumpt, F. (1965). *Myiasis in man and animals in the Old World: A textbook for physicians, veterinarians, and zoologists*. Butterworths, London, UK. 267 pp.