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Research



# Accidental Nasal Myiasis Caused by *Megaselia rufipes* (Diptera: Phoridae) in a Child

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## Abstract

A case of a nasal myiasis in a 3-yr-old Italian girl who was referred to Bambino Gesù Hospital in Rome, Italy, is reported. Larvae discharged with the nasal mucus were microscopically identified as *Megaselia* spp.; DNA barcoding analysis showed that they belonged to the 'scuttle fly' species *Megaselia rufipes* (Meigen). Based on the patient's history, she became infected when she played outside. This is the first report of myiasis in humans due to *M. rufipes* (Diptera: Phoridae).

**Key words:** accidental myiasis, child, Phoridae, *Megaselia rufipes*

There are two main systems for categorizing myiasis—i.e., the infestation of live vertebrate animals with dipterous larvae: firstly, in relation to the anatomical location of the infestation, on or in the host, i.e., as cutaneous, auricular, nasopharyngeal, ophthalmic, gastrointestinal, and urogenital; or, secondly, on the basis of the parasite–host relationship, i.e., as accidental, facultative, or obligatory (Hall and Smith 1993, Scholl et al. 2009).

Members of the family Phoridae represent one of the largest families in the order Diptera and are commonly known as 'scuttle, humpback, or coffin' flies, although the latter name is applied mainly to the species *Conicera tibialis* (Martín-Vega et al. 2011). These flies can exploit a large variety of environmental and ecological niches and exhibit a greater larval habitat diversity compared with other insects. They are distributed worldwide with approximately 245 described genera and over 3,000 species (Disney 1989, 1994). In this family, the cosmopolitan genus *Megaselia* includes over 1,400 species showing a wide variety of eco-biological characteristics including diet. Many reports show that the larvae of some species of *Megaselia* can parasitize humans and be causal agents of myiasis (Zumpt 1965, Hall and Smith 1993) or are otherwise harmful to human health as vectors of pathogens (Disney 1994).

Cases of intestinal, ocular, and urogenital myiasis have been reported worldwide; however, the species most frequently involved is *Megaselia scalaris* (Disney 2008). We describe herein a recent case of accidental myiasis caused by phorid fly larvae in Italy, the first reported for the species *Megaselia rufipes*.

## Materials and Methods

A 3-yr-old girl living in a rural area near Sora, Province of Rome, was presented to the Emergency Room of Bambino Gesù Pediatric Hospital in Rome with a history of fever, persistent nose, and pharynx itch followed by the nasal discharge of mucus containing two living larvae, which were collected by the girl's mother. After the otorhinolaryngological evaluation was carried out in the Emergency Room, hospitalization was arranged for in-depth investigations. Hence, the patient was transferred to the General Pediatrics Unit due to suspected helminthiasis and the larvae were brought to the Parasitology Unit for a preliminary observation. The larvae were put in a tube (without any liquid) and sent for detailed investigations and identification to the Parasitology Unit of the University of Foggia, and soon after their arrival they were observed under a stereomicroscope (Discovery, V12 Zeiss, Oberkochen, Germany).

Genomic DNA was extracted from one of the specimens using the Nucleospin Tissue kit (Macherey-Nagel, Amsterdam, The Netherlands) according to the manufacturer's instructions. The extracted DNA was stored at –20°C until polymerase chain reaction

(PCR) amplification was performed. All PCR amplifications were performed in a 2720 Thermal Cycler T3000 (Applied Biosystems, Foster City, California, USA). An approximately 710-bp gene fragment of the *cox1* (cytochrome *c* oxidase subunit I) gene-based DNA barcode was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994).

Amplification was performed in a total reaction volume of 25 µl containing 10 µl of REDTaq ReadyMix PCR Reaction Mix (Merck, Darmstadt, Germany), 1 µl of each primer, 8 µl of water, and 5 µl of genomic DNA. The PCR protocol was as follows: 1 min at 94°C followed by five cycles of 94°C for 1 min, 45°C for 1.5 min, and 72 °C for 1.5 min followed by 35 cycles of 94°C for 1 min, 50°C for 1.5 min, and 72°C for 1 min with a final extension step of 72°C for 8 min, according to Boehme et al. (2010). A negative control (PCR-grade water) was included in each PCR run. The PCR products were run on 2% agarose gel.

Purification and sequencing of PCR products with the above-described universal primers (in both forward and reverse directions) were performed by Eurofins MWG Operon (Ebersberg, Germany). The sequences generated from the one specimen were edited and aligned manually using BioEdit Sequence Alignment Editor v7.2.5 (Hall 1999) and compared with *M. rufipes* DNA barcode sequences available in GenBank database using the BLASTn program (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). The most representative sequence was submitted to GenBank.

Phylogenetic analyses were conducted in Geneious version 2020.1 (https://www.geneious.com) using the Neighbor-Joining algorithm. The evolutionary distances were computed using the Tamura–Nei method and bootstrap confidence assessed with 1,000 replicates. The analysis involved nine nucleotide sequences. The tree was rooted using a *Lipoptena cervi* (Diptera: Hippoboscidae) sequence as the outgroup (accession number: MN370847).

During her hospitalization, the girl's clinical conditions remained stable with normal hematochemical values. She was subjected to several nasal cleansing procedures with physiological solution, but no further larvae were recovered. A fiber optic laryngoscopy was carried out, but no larvae or foreign bodies were found in the nostrils, nasopharynx, larynx, or at the root of the tongue. She was precautionarily treated against helminthiasis with albendazole for 3 d. The girl was then discharged from the hospital with no further therapy and without any specific symptoms. Her mother was asked to refer to her pediatrician if any additional assistance was required.

## Results

Under the light microscope the two specimens appeared light brown, 3.5 mm long × 1.5 wide mm and dorsoventrally flattened; the respiratory horns were missing as they had broken off due to their brittleness. They were both identified morphologically as *Megaselia* spp. (Diptera: Phoridae) at the pupal stage (Fig. 1). They did not differ from published images of *M. rufipes* puparia (e.g., Smith 1986, Ferrar 1987; https://sites.google.com/site/dipterabrachyceracyclorrhapha/suborder-brachycera/phoridae-scuttle-fly/genus-megaselia/megaselia-rufipes), but most species of *Megaselia* have not been described in their immature stages so, based on morphology alone, we could not definitively exclude the possibility that they were of another species. The molecular BLASTn analysis showed that the sequence gave a 100% match with *M. rufipes* (Diptera: Phoridae) and all sequences of *M. rufipes* included in the phylogenetic tree (Boehme et al. 2010), and compared with our sequence, showed identical nucleotide sequences except slight differences in 1–2 bp. Therefore, the intraspecific variation did not exceed 1%. The nucleotide divergence ranged from 9.8 to 11.4% from closely related species included in the analysis (*M. lata* and *M. scalaris*). The distance matrix is shown in Table 1. The sequence obtained from our specimen was submitted to GenBank under accession number: MT472135 (Fig. 2).

## Discussion

Adult morphological identification is considered the 'gold standard' for the identification of Diptera species. However, because the specimens were dead it was not possible to rear them to the adult stage. Robustness of the *cox1* gene as a diagnostic marker for identification of *Megaselia* has been previously demonstrated, as this genetic marker allowed the identification of *M. rufipes* in Fig. 2, among Phoridae flies in the field of forensic entomology (Boehme et al. 2010).

In the present study, the involvement of *M. rufipes* was confirmed with 100% confidence from the specimen sequenced. Thus, the combination of morphological (at least to the genus level) and molecular observation allowed the identification of *M. rufipes* as responsible for this case of accidental nasal myiasis.

*Megaselia rufipes*—which probably originated in Europe (Disney 2009)—exploits a wide range of mainly decaying, organic materials


**Fig. 1.** Image of *Megaselia* sp. under the stereomicroscope: puparium with the pair of pupal respiratory horns broken off.

**Table 1.** Absolute nucleotide differences between the *Megaselia rufipes* specimen investigated in the present study and *Megaselia* sequences included in the phylogenetic tree, available from GenBank for the analyzed *cox1* region

| | GU075403 *M. rufipes* | GU075404 *M. rufipes* | GU075405 *M. rufipes* | GU075406 *M. rufipes* | JN896298 *M. scalaris* | KP693245 *M. lata* | KR654299 *M. scalaris* | MN370847 *L. cervi* | MT472135 *M. rufipes** |
|---|---|---|---|---|---|---|---|---|---|
| GU075403 *M. rufipes* | | 1 | 2 | 1 | 65 | 56 | 64 | 83 | 1 |
| GU075404 *M. rufipes* | 1 | | 1 | 2 | 64 | 55 | 63 | 82 | 0 |
| GU075405 *M. rufipes* | 2 | 1 | | 3 | 63 | 56 | 64 | 81 | 1 |
| GU075406 *M. rufipes* | 1 | 2 | 3 | | 64 | 55 | 65 | 82 | 2 |
| JN896298 *M. scalaris* | 65 | 64 | 63 | 64 | | 56 | 6 | 97 | 64 |
| KP693245 *M. lata* | 56 | 55 | 56 | 55 | 56 | | 60 | 86 | 58 |
| KR654299 *M. scalaris* | 64 | 63 | 64 | 65 | 6 | 60 | | 101 | 67 |
| MN370847 *L. cervi* | 83 | 82 | 81 | 82 | 97 | 86 | 101 | | 83 |
| MT472135 *M. rufipes** | 1 | 0 | 1 | 2 | 64 | 58 | 67 | 83 | |

*The sequence obtained from our specimen.

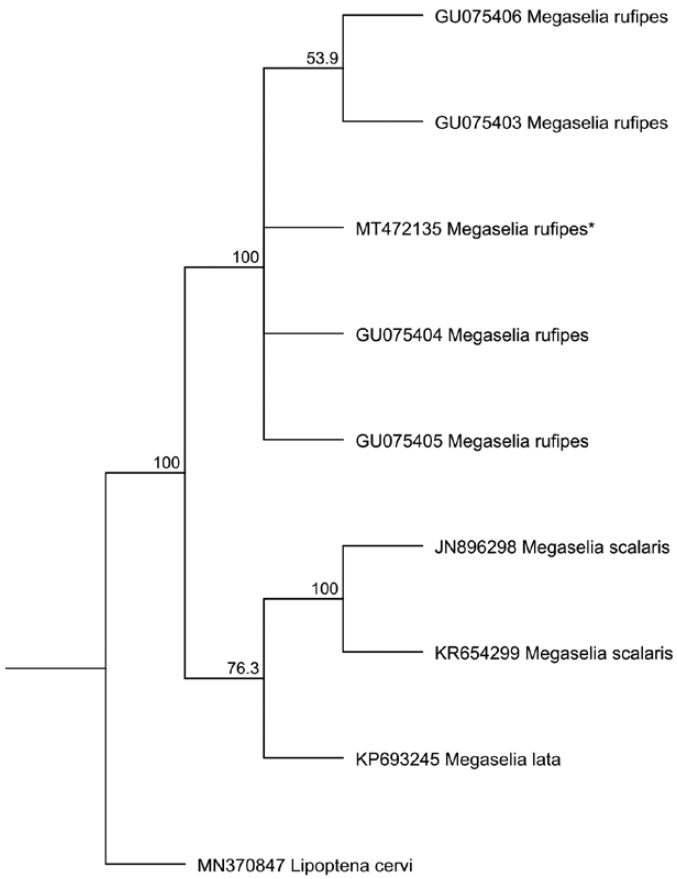


**Fig. 2.** Neighbor-Joining tree. Relationships between the *Megaselia rufipes* specimen investigated in the present study and *Megaselia* sequences available from GenBank are presented based on the partial cytochrome *c* oxidase subunit I (*cox1*) gene fragment. Labels include accession numbers and species identity. The haplotype identified from the present study is labeled with its GenBank accession number (MT472135), species identity, and marked with an asterisk (*). Bootstrap values (percentages of 1,000 replications) are indicated at the internodes. *Lipoptena cervi* (Diptera: Hippoboscidae) was used as outgroup.

for food at the larval stage, that include: decaying vegetation, vertebrate excrements (swallows, humans, bats), dead insects, vertebrate carrion, but also some human foods such as old cheese milk (Disney 1994). Records of its distribution are being continuously extended (e.g., Gonzalez-Vainer et al. 2012), perhaps because it is frequently overlooked or misidentified.

*Megaselia rufipes* is also of great forensic significance (Disney 2011) and has been frequently reported in human corpses laid on the ground (Disney 1994). However, as an example of the diversity of its behavior, *M. rufipes* has also been recently identified as a facultative parasitoid in honey bees, *Apis mellifera*, in northern Italy (Dutto and Ferrazzi 2014).

Although other species of *Megaselia* are also regarded as species of forensic interest (Feng and Liu 2013, 2014), *M. scalaris* is the species most frequently recorded in cases of human and/or animal myiasis, i.e., urogenital (Solgi et al. 2017), intestinal (Udgaonkar et al. 2012), nasopharingeal (Hira et al. 2004), and ocular (Diclaro et al. 2011), while *M. spiracularis* has been reported on one occasion as an agent of pulmonary myiasis (Komori et al. 1978).

As to the involvement of *M. rufipes* in myiasis, in the literature, James (1947)—later repeated by Zumpt (1965)—in the section '*Megaselia rufipes* (Meigen)' cites Patton (1922), stating that '*Patton has recorded two cases of wound myiasis in cattle in India caused by this species, one of them in association with* M. scalaris'. Then, James (1947) adds: '*It may easily be a human parasite at times. Supposed cases of intestinal myiasis have been recorded, but evidence is not conclusive*'.

Thus, this is to our knowledge the first conclusive report ever of myiasis in humans due to *M. rufipes*. This kind of myiasis falls within the categorization of accidental, based on parasite–host relationships, as it is reasonable to hypothesize that, playing outside on ground contaminated with decomposing material, the girl accidentally aspirated the larvae of unknown instar, which remained in the nasal passages for some days causing the symptoms described above. They would have grown rapidly (possibly even moulting from an immature instar) due to the high nasal temperature, from a mean of 30.2 ± 1.7°C to 34.4 ± 1.1°C depending on the site and respiration cycle (Lindemann et al. 2002), much higher than the temperatures of 20°C to 23°C used in the laboratory to study development of this species (Disney 2005). Pupariation must have occurred after the mother collected the ejected larvae and brought them to the hospital.

This report confirms the importance of recording cases of myiasis and correctly identifying the causative agents, which enables clinicians to highlight the risk factors in the social and hygienic situations in which the patients live. In this specific case, the identification of the phorid species represented an opportunity to make parents, relatives, and local pediatricians aware of the risks of the unsanitary environment the little girl was exposed to. As this is the first report of *M. rufipes* myiasis it is clearly a very rare event and, therefore, the lack of parent/doctor awareness of the potential risk in this case is understandable. With the relatively mild symptoms, it cannot be excluded that similar cases might have gone undetected in the past.

## Ethics

The child's parents were informed of the study objectives and gave written informed consent for the case description and samples to be used for analysis.

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