FORENSICALLY IMPORTANT MUSCIDAE (DIPTERA) ASSOCIATED WITH DECOMPOSITION OF CARCASSES AND CORPSES IN THE CZECH REPUBLIC

VANDA KLIMESOVA[1], TEREZA OLEKSAKOVA[1], MIROSLAV BARTAK[1], HANA SULAKOVA[2]

[1]Department of Zoology and Fisheries
Czech University of Life Sciences Prague (CULS)
Kamycka 129, 165 00 Prague 6 – Suchdol
[2]Institute of Criminalistics Prague (ICP)
post. schr. 62/KUP, Strojnicka 27, 170 89 Prague 7

CZECH REPUBLIC

klimesovav@af.czu.cz

Abstract: In years 2011 to 2015, three field experiments were performed in the capital city of Prague to study decomposition and insect colonization of large cadavers in conditions of the Central Europe. Experiments in turns followed decomposition in outdoor environments with the beginning in spring, summer and winter. As the test objects a cadaver of domestic pig (*Sus scrofa* f. *domestica* Linnaeus, 1758) weighing 50 kg to 65 kg was used for each test. Our paper presents results of family Muscidae, which was collected during all three studies, with focusing on its using in forensic practice. Altogether 29,237 specimens of the muscids were collected, which belonged to 51 species. It was 16.6% (n = 307) of the total number of Muscidae family which are recorded in the Czech Republic. In all experiments the species *Hydrotaea ignava* (Harris, 1780) was dominant (spring = 75%, summer = 81%, winter = 41%), which is a typical representative of necrophagous fauna on animal cadavers and human corpses in outdoor habitats during second and/or third successional stages (active decay phase) in the Czech Republic.

Key Words: Muscidae, Diptera, forensic entomology, pyramidal trap

INTRODUCTION

Forensic or criminalistic entomology is the science discipline focusing on specific groups of insect for forensic and law investigation needs (Eliášová and Šuláková 2012). Its main principle is to determinate the minimum time since death or so called post mortem interval (PMI) by establishing the time of colonization (or the time of the first oviposition) of a corpse by insects. Forensic entomology can also provide evidence whether the body was manipulated at the crime scene (e.g., after buried) or moved from a location to other as well as when the body was subsequently moved and it's still missing by genetic analysis of found larvae DNA profile of the host and therefore his/her identity can be obtained (Amendt et al. 2011). Forensic entomology with help of studies results can be consider as very effective and often crucial tool in explaining of criminal cases (Martins et al. 2013).

Muscidae is a large family of order Diptera with worldwide distribution. The family has about 4,500 species in 180 genera in the word (De Carvalho et al. 2005) and 562 species in 44 genera are known from Europe (Pont 2005). In the Czech Republic, 307 species is listed (Barták 2013). Several species of muscids are ubiquitous and synanthropic and therefore of medical importance because of their relationship with man. The synantropic habits ensure that some species are likely to become involved in medical and forensic cases (Byrd and Castner 2010, Smith 1986). Common members of this family, which can visit and colonized a body soon after death, are *Musca domestica* Linnaeus, 1758 and *Musca autumnalis* DeGeer, 1776 (Daněk 1990, Gennard 2012) or members of genus *Muscina* and *Hydrotaea* (Eliášová and Šuláková 2012, Šuláková 2014). Muscid flies tend to arrive at corpses after the blow flies (Calliphoridae) or later after the flesh flies (Sarcophagidae) (Byrd and Castner 2010, Eliášová and Šuláková 2012, Šuláková 2014). Muscid females often lay eggs in natural body openings, at wound, or blood-soaked clothing (Byrd and Castner 2010) as well as beneath the body into soil soaked by

decomposition liquid (Šuláková 2014). Larvae usually feed directly on the carrion but can also pray on the eggs and larvae of other necrophagous flies (Byrd and Castner 2010, Šuláková 2014) and in this way may affect the faunal composition (Byrd and Castner 2010).

MATERIAL AND METHODS

Description of the locality field experiments

Summer experiment

The summer experiment was situated in a fenced ground of the Police school in Prague 9 – Hrdlořezy, the eastern suburb of the capital. The research site consisted of bushy area with smaller, sunlit openings with grass and deciduous trees in vicinity. Geographic coordinates: 50°5'22" N, 14°30'19" E; altitude: 240 m amsl.

For purposes of the summer experiment, domestic pig (*Sus scrofa* f. *domestica*) weighing about 65 kg was killed by a single shot to the front of its head with a 0.22 calibre rifle. The cadaver was moved the trial site within 20 minutes, foil-wrapped during transport to avoid any earlier oviposition. On the experimental area carcasses was dressed in human clothing to more accurately mimic the decay of the human body. According Daněk (1990) clothing, among other factors, affects the rate of decomposition, acts in part as a shield. The experimental animal had a bleeding wound imitating injuries of a murder victim. The experiment lasted from 13 July 2011 and was completed 18 October 2012. Insects of the cadaver and its immediate surroundings were captured using am entomological net, tweezers and pitfall traps filled with a mixture of saline water with added detergent. Collection of insects was different according to the time from the start of the experiment: 1st to 17th day exposure was conducted trapping insects once a day, 17th to 62nd day every two to three days, 62nd to 195th day, once every ten to fourteen days and 195th to 464th day only once a month.

Spring experiment

The spring experiment was situated in a fenced experimental field of the Czech University of Life Sciences in Prague – Troja, the northern part of the capital. The area was on a west-facing slope near Vltava River, in a flooding zone, on grass-covered, sunlit opening with fruit trees and bushes around. Geographic coordinates: 50°7'16" N, 14°23'53" E; altitude: 185 m amsl.

For the spring experiment, domesticated pig weighing about 53 kg was used, which have died naturally 19 March 2012, the day before the start of the experiment. During the subsequent transfer on the experimental site the pig was placed in a sealed plastic bag to prevent earlier colonization by insects. This cadaver was also dressed in clothing. For collecting of the adults of the family Muscidae, which arrived on the cadaver or hatched on it, a pyramid trap was used. The trap consisted of soft polyester fabric placed over the carcasses shaped bottom open oblique pyramid (description Barták and Roháček 2011) which collects insect 24/24. The trap had base dimensions 2 m x 2 m to overlie the entire experimental animal, and has been placed 20–40 cm over the ground or vegetation, in order not to prevent access of insects to a test object. On the top of the trap, a collecting plastic bottle of capacity of 5 litres was placed and filled by a killing and preservative solution with its composition: 1.5 l of water, 2 ml 36–38% of formaldehyde and 1 ml of detergent. Experiment started 20 March 2012 by free exposition of cadaver and 6 June 2013 was terminated prematurely for reasons of flooding and damaging to the experimental area by the swollen Vltava River during the floods in Prague in 2013. Contents of the sampling vessel on the pyramid traps were collected at the following intervals: 1st–197th day of exposure once a week, 197th to 267th day once every two weeks, 267th to 393rd day once a month, and after two weeks until the end experiment.

Winter experiment

The winter experiment was also situated in a fenced experimental field of the Czech University of Life Sciences in Prague – Troja, on the west-facing slope near Vltava River, above the flooding zone, on sand-covered, sunlit opening among fruit trees. Geographic coordinates: 50°7'14.5" N, 14°23'56" E; altitude: 190 m amsl.

In the free exposition, the next dead domesticated pig weighing about 50 kg has been exposed. The pig was killed by a veterinarian by injection a month before the study and frozen to imitate a frozen

overdosed drug user. For the purpose, the carcass was dressed to men's shirts, sweatpants and socks. The winter experiment has been running from 9 December 2014 and has not been finished yet. For the purposes of this study, results of family Muscidae since the beginning, 9 December 2014, to 31 December 2015 are only presented. Trapping method coincides with the spring experiment, by the pyramidal trap. Samples were taken at the following intervals: at the beginning of the experiment, i.e. from December 2014 were sampled once per month. From January to early March 2015 every 14 days, from March samples were collected once a week until October. In October, the sampling interval is extended again for 14 days.

Material

The material was determined to species by Lt-Col. Ing. Hana Šuláková, Ph.D., Bc. Markéta Slobodová and prof. RNDr. Miroslav Barták, CSc. according to the monograph The Muscidae (Diptera) of Central Europe (Gregor a kol. 2002).

RESULTS

Summer

In the summer experiment, the total of 234 representatives of the family Muscidae belonging to these 8 species were collected and determined. These species, listed in alphabetical order, were: *Graphomya maculata* (Scopoli, 1763), *Hydrotaea armipes* (Fallen, 1825), *Hydrotaea dentipes* (Fabricius, 1805), *Hydrotaea ignava, Hydrotaea meteorica* (Linnaeus, 1758), *Hydrotaea pilipes* (Stein, 1903), *Muscina prolapsa* (Harris, 1780) and *Thricops simplex* (Wiedemann, 1817) (Figure 1). The first four stages of decomposition were: fresh 13 July to 14 July 2011, bloated 15 July to 16 July 2011, active decay 17 July to 1 August 2011, and dry remains 3 August to 12 September 2011.

Spring

From the obtained material of the field spring experiment, 19,910 specimens family Muscidae belonging to 38 species were collected and determined. The following species were recorded (in alphabetical order): *Azelia cilipes* (Haliday, 1838), *Azelia trigonica* (Hennig, 1956), *Azelia triquetra* (Wiedemann, 1817), *Azelia zetterstedti* (Rondani, 1866), *Coenosia humilis* (Meigen, 1826), *Coenosia nigridigita* (Rondani 1866), *Coenosia testacea* (Robineau–Desvoidy, 1830), *Coenosia tigrina* (Fabricius, 1775), *Graphomya maculata, Hebecnema nigra* (Robineau–Desvoidy, 1830), *Hebecnema umbratica* (Meigen, 1826), *Hebecnema vespertina* (Fallen, 1823), *Helina impuncta* (Fallen, 1825), *Helina Latitarsis* (Ringdahl 1924), *Helina reversio* (Harris, 1780), *Helina sexmaculata* (Preyssler, 1791), *Hydrotaea aenescens* (Wiedemann, 1830), *Hydrotaea armipes, Hydrotaea cyrtoneurina* (Zetterstedt, 1845), *Hydrotaea dentipes, Hydrotaea floccosa* (Macquart, 1835), *Hydrotaea ignava*, *Hydrotaea meteorica*, *Hydrotaea pilipes*, *Hydrotaea similis* (Meade, 1887), *Limnophora nigripes* (Robineau–Desvoidy, 1830), *Lispe tentaculata* (De Geer, 1776), *Musca stabulans* (Fallen, 1817), *Muscina levida* (Harris, 1780), *Muscina pascuorum* (Meigen, 1826), *Muscina prolapsa, Mydaea ancilla* (Meigen, 1826), *Mydaea Corni* (Scopoli, 1763), *Mydaea urbana* (Meigen, 1826), *Myospila meditabunda* (Fabricius, 1781), *Phaonia subventa* (Harris, 1780), *Potamia littoralis* (Robineau–Desvoidy, 1830) and *Thricops simplex* (Figure 1). The first four stages of decomposition were: fresh 20 March to 27 March 2012, bloated 3 April to 24 April 2012, active decay 2 May to 21 August 2012, and dry remains 28 August to 11 December 2012.

Winter

During the winter experiment, 9,093 specimens of the family Muscidae, belonging up to 41 species were capture and determined. The following species were recorded (in alphabetical order): *Azelia nebulosa (*Robineau–Desvoidy, 1830), *Azelia triquetra, Coenosia atra (*Meigen, 1830), *Coenosia humilis, Coenosia infantula (*Rondani, 1866), *Coenosia rufipalpis (*Meigen, 1826), *Coenosia testacea, Coenosia tigrina, Eudasyphora zimini* (Henning, 1963), *Graphomya maculate, Gymnodia humilis* (Zetterstedt, 1860), *Hebecnema vespertina, Helina depuncta* (Fallén, 1825), *Helina impuncta, Helina lasiophthalma* (Macquart, 1835), *Helina reversion, Helina setiventris* (Ringdahl, 1924), *Hydrotaea aenescens (*Wiedemann, 1830), *Hydrotaea armipes, Hydrotaea dentipes, Hydrotaea floccose, Hydrotaea ignava, Hydrotaea pilipes, Hydrotaea similis, Musca autumnalis* (De Geer, 1776), *Musca domestica* (Linnaeus, 1758), *Musca osiris* (Wiedemann, 1830), *Muscina levida, Muscina pabulorum*

(Fallén 1817), *Muscina prolapse, Muscina stabulans, Mydaea ancilla, Mydaea corni, Myospila meditabunda, Phaonia errans* (Meigen, 1826), *Phaonia subventa, Phaonia trimaculata* (Bouché, 1834), *Phaonia tuguriorum* (Scopoli, 1763), *Pyrellia vivida* (Robineau–Desvoidy, 1830), *Stomoxys calcitrans* (Linnaeus, 1758) and *Thricops simplex* (Figure 1). The first four stages of decomposition were: fresh 9 December 2014 to 3 March 2015, bloated 18 March to 1 April 2015, active decay 7 April to 1 September 2015, and dry remains 9 September to 8 December 2015.

Figure 1 Compared frequency of occurrence of muscids in all experiments

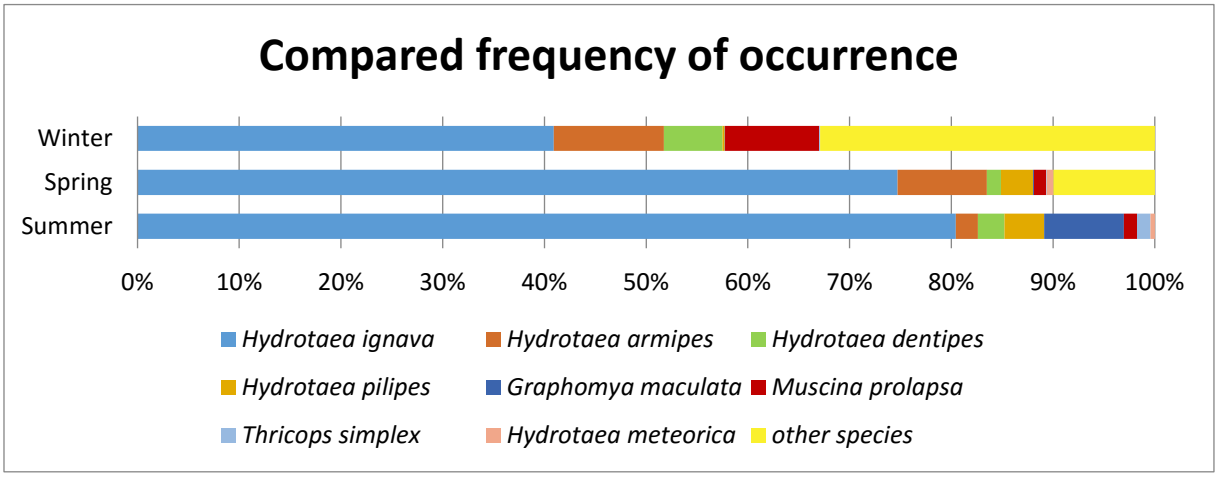


Figure 2 Summer – Quantity of three most common species according to decay phases

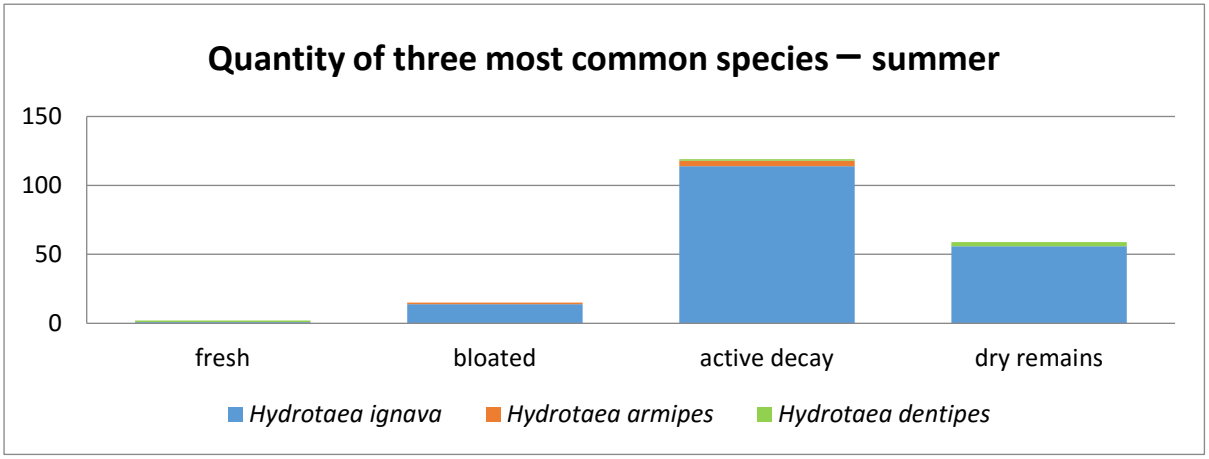


Figure 3 Spring – Quantity of three most common species according to decay phases

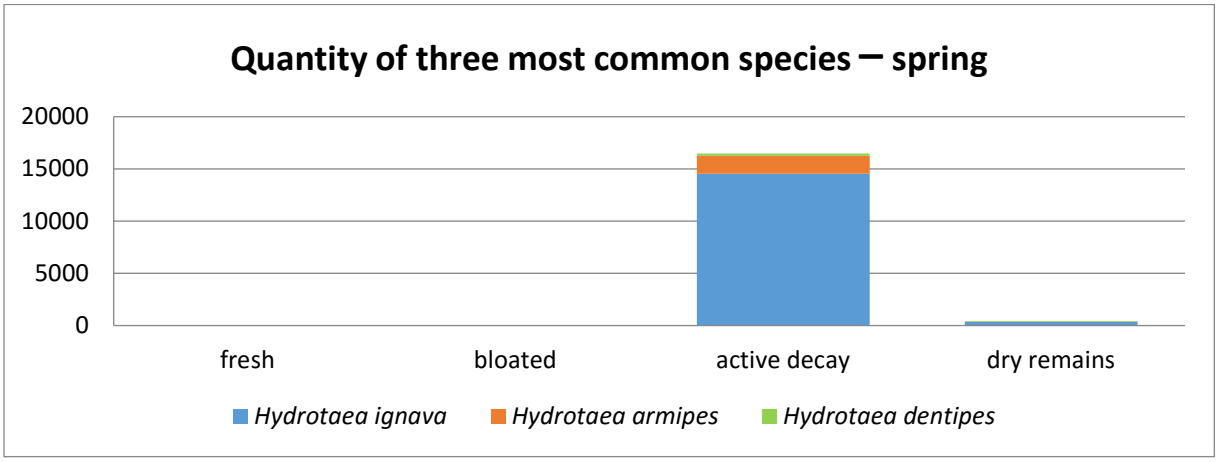
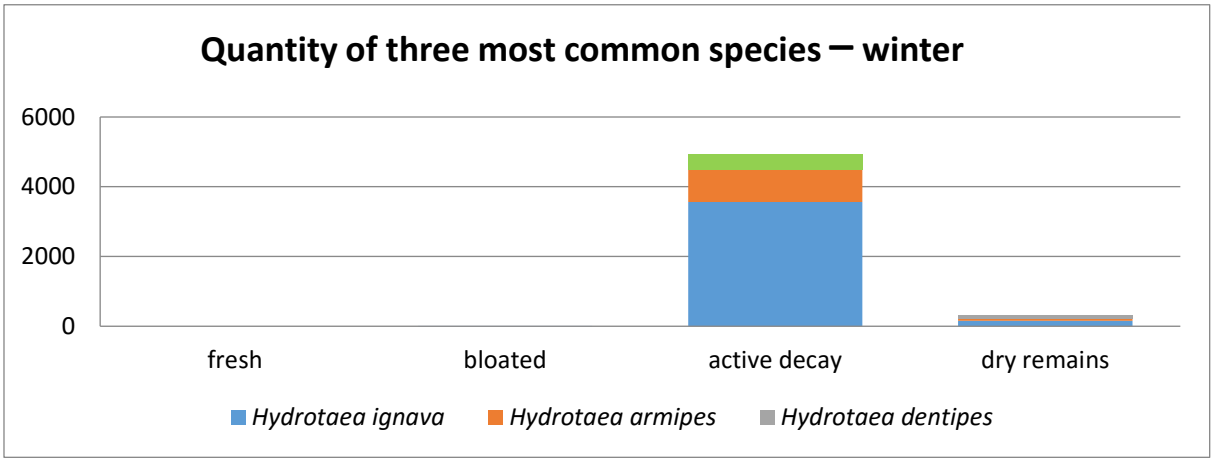


Figure 4 Winter – Quantity of three most common species according to decay phases



CONCLUSION AND DISCUSSION

During the experiments have been recorded 51 species of families Muscidae, which are 16.6% (n = 307) of the total number of species that can occur in the Czech Republic.

The most numerous species was *Hydroatea ignava*. Its abundance and importance discusses Daněk (1990) and Sukantson (2007) who confirm that 70% of all individuals detained on the corpse may be just *Hydrotaea ignava*. The most frequent species of the genus *Hydrotaea*, namely *Hydrotaea ignava*, *Hydrotaea armipes* and *Hydrotaea dentipes*, were related to third stage of decomposition, the active decay phase, in all three trials (see Figure 2, Figure 3 and Figure 4). These statements respond to published data (e.g., Byrd and Castner 2010, Eliášová and Šuláková 2012, Šuláková 2014) as well as our findings on human corpses in outdoor conditions (Šuláková, unpublished data). Other abundant species on the carcase of experiment Spring were also *Hydrotaea pilipes* and *Hydrotaea floccosa* – the literature dealing with forensic entomology not mention about them. The reason could be that species of *Hydrotaea pilipes* has been often confused with the species *Hydrotaea ignava* (females are very similar) and species *Hydrotaea floccosa* was probably due to poor recognisability confused with the species of *Hydrotaea armipes* (Klimešová et al. 2014).

Musca autumnalis and *Musca domestica*, which are usually mentioned as forensically important muscids, especially for the first or the second succession wave (Daněk 1990, Gennard 2012, Smith 1986), were not involved in Spring and Summer experiments at all, although, during Spring and Winter experiment (in Troja), near zoological garden and its stables were a potential source of them. In experiment Winter, there were present only in a small amount from the second to the beginning of the fourth stage of decomposition. We suppose that the occurrence of these species was probably caused by different (slower) processes of decomposition due to the beginning of the experiment in winter. Similar patterns are observed on human corpses in the Czech Republic; *Musca autumnalis* and *Musca domestica* are rare on dead bodies and their oviposition is usually qualified by both their presence in the locality and the appearance of faeces on or near the body (Šuláková, unpublished data). The result reflect that the genus *Muscina*, mainly *Muscina prolapsa*, which was recorded in all three trials but mostly in Winter (Figure 1), is more common and important for forensic practise then the genus *Musca*. These data confirm our observations from the human corpses, when muscids of the genus *Muscina* colonize dead bodies more frequently during winter, what time their make the best of reduction or absence of the usual first colonizers from the family Calliphoridae (Šuláková, unpublished data).

According our results, occurrence, frequency, and quantity of muscids are not affected by the reason of the death of the object (e.g., bodies with or without bleeding wounds). The family is associated mainly with third stage of decomposition. Nevertheless seasons can partly influence the species composition of muscids and the time their staying on the body.

In smaller amounts, many species that are commonly not found on corpses were recorded, for example: *Coenosia humilis, Coenosia testacea, Helina impuncta, Mydaea urbana* or *Azelia cilipes*. Their larvae are commonly feeding and growing on different types of decaying organic material, e.g.,

manure, meat, plants, and we assume they use carcases only occasionally. Some of them are not strictly necrophagous but predatory (the genus *Coenosia* are predatory also like adults). *Azelia trigonica*, recorded in twenty specimens in May 2012 and July 2012 in Spring experiment, is guided on the red list of endangered species of the Czech Republic as "endangered" (Farkač et al. 2005).

ACKNOWLEDGEMENTS

This paper was supported by the project No. VF20152015041 of Ministry of the Interior of the Czech Republic (HŠ) and by S grant of MSMT (Ministry of Education, Sports and Youth) (MB). The authors gratefully thank the Police school in Prague – Hrdlořezy and Ing. Josef Sus, CSc. from the Czech University of Life Sciences in Prague – Suchdol for providing the experimental fields.

REFERENCES

Amendt, J., Richards, C.S., Campobasso, C.P., Zehner, R., Hall, M.J.R. 2011. Forensic entomology: applications and limitations. *Forensic Science, Medicine and Pathology.* 7(4): 379–392.

Arnaldos, M.I., Romera E., Presa J.J., Luna A., García M.D. 2004. Studies on seasonal arthropod succession on carrion in the southeastern Iberian Peninsula. *Internet J. Legal. Med.* 118: 197–205.

Barták, M., Vaněk, J., Hlavová A., Hlava, J. 2013. Mouchovití (Diptera, Muscidae) české části Krkonoš. *Opera Corcontica*, 50: 151–170.

Barták, M., Roháček, J. 2011. Records of interesting flies (Diptera) attracted to meat baited pyramidal trap on sapping stump of European walnut (Juglans regia) in Central Bohemia (Czech Republic). *Časopis slezského zemského muzea* (A) 60(3): 223–233.

Byrd, J.H., Castner, J.L. 2010. *Forensic entomology: the utility of arthropods in legal investigations*. CRC Press.

Daněk, L., 1990. Možnosti využití entomologie v kriminalistice. *Čs. Kriminalistika*. 1: 47–49.

De Carvalho, C.J.B., Couri, M.S., Lopes, S.M., Pamplona, D., Pont, A.C. 2005. *A catalogue of the Muscidae (Diptera) of the Neotropical Region*. Magnolia Press.

Farkač, J., Král, D., Škorpík, M. 2005. *Červený seznam ohrožených druhů České republiky Bezobratlí*. Agentura ochrany přírody a krajiny ČR.

Eliášová, H., Šuláková, H. 2012. *Forenzní biologie. In: Štefan, J., Hladík, J. a kol. Soudní lékařství a jeho moderní trendy*. Grada Publishing.

Gennard, D.E. 2012. Forensic Entomology: an inntroduction – second edition. Wiley–Blackwell.

Gregor, F., Rozkošný, R., Barták, M., Vaňhara, J. 2002. The Muscidae (Diptera) of Central Europe. Folia Fac. Sci. Nat. Univ. Masaryk.

Klimešová, V., Slobodová M., Šuláková H., Barták M. 2014. Využití čeledi Muscidae (Diptera) ve forenzní praxi. *6th Workshop on biodiversity, Jevany.* Česká zemědělská univerzita v Praze.

Martins, G., dos Santos, W.E., Creao–Duarte, A.J., da Silva, L.B.G. Oliveira, A.A.F. 2013. Estimate of postmortem interval through forensic entomology in a canine (Canis lupus familiaris Linnaeus 1758) in Cabedelo–PB, Brazil: case report. *Arquivo brasileiro de medicina veterinaria e zootecnia*, 65(4): 1107–1110.

Pont, A.C. 2005. *Fauna Europaea: Muscidae*. In Pape T. (ed.): *Fauna Europaea: Diptera, Brachycera. Fauna Europaea version 1.2.* Available at: http://www.faunaeur.org. [2016-09-01].

Smith, K.G.V. 1986. *A manual of forensic entomology*. British Museum (Natural History) and Cornell University.

Sukontason, K., Narongchai, P., Kanchai, Ch., Vichairat, K., Sribanditmongkol, P., Bhoopat, T., Kurahashi, H., Chockjamsai, M. 2007. Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. *Parasitology Research.* 101(5): 1417–1423.

Šuláková, H. 2014. Forenzní entomologie – když smrt je začátek. *Živa*. 5: 250–256.